Twenty years of research in ecosystem functions in aquatic microbial ecotoxicology

Soizic Morin, Joan Artigas

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3 Soizic Morin and Joan Artigas

4

5 **Abstract**

6 One of the major threats to freshwater biodiversity is water pollution including excessive loads of
7 nutrient, pesticides, industrial chemicals, and/or emerging contaminants. The widespread use of
8 organic pesticides for agricultural and non-agricultural (industry, gardening, etc…) purposes has
9 resulted in the presence of their residues in various environments, including surface waters. However,
10 the contribution of pesticides to the deterioration of freshwater ecosystems (i. e. biodiversity decline
11 and ecosystem functions impairment) remains uncertain. Once in the aquatic environment,
12 pesticides and their metabolites can interact with microbial communities causing undesirable effects.
13 The existing legislation on ecological quality assessment of water bodies in Europe is based on water
14 chemical quality and biological indicator species (Water Framework Directive, Pesticides Directive)
15 while biological functions are not yet included in monitoring programs. In this literature review, we
16 analyse the last twenty years (2000-2020) of research on ecological functions provided by
17 microorganisms in aquatic ecosystems. We describe the set of ecosystem functions investigated in
18 these studies and the range of endpoints used to establish causal relationships between pesticide
19 exposure and microbial responses. We focus on studies addressing the effects of pesticides at
20 environmentally realistic concentrations and at the microbial community level to inform the
21 ecological relevance of the ecotoxicological assessment. This literature review highlights that most
22 studies were performed using benthic freshwater organisms, and that autotrophic and heterotrophic
23 communities are most often studied separately, usually testing the pesticides that target the main
24 microbial component (i. e. herbicides for autotrophs and fungicides for heterotrophs). Overall, most
25 studies demonstrate deleterious impacts on the functions studied, but this review points to the
26 following shortcomings: i) the non-systematic analysis of microbial functions supporting aquatic
27 ecosystems functioning, ii) the study of ecosystem functions (i. e. nutrient cycling) via proxies (i. e.
28 potential extracellular enzymatic activity measurements) which are sometimes disconnected from
29 the current ecosystem functions, and iii) the lack of consideration of chronic exposures to assess
30 impact, adaptations or recovery of aquatic microbial communities to pesticides.

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35 **Running head**: Ecosystem functions in aquatic microbial ecotoxicology

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1. Introduction

Aquatic microbial ecotoxicology (AME) is a research subject at the interface between aquatic microbial ecology and ecotoxicology (Ghiglione et al. 2016). In aquatic ecosystems, microbial communities cover a large diversity of microorganisms, living forms (planktonic/benthic, solitary/colonial...), and functionalities. They are composed of autotrophic (including cyanobacteria, green algae and diatoms, among others) and heterotrophic microorganisms (archaea, bacteria, fungi and protozoa) and play a pivotal role in global biogeochemical cycles, nutrients cycles and energy flow in aquatic ecosystems (Lock et al. 1984, Battin et al. 2003). One of the major goals of AME is to improve mechanistic understanding of ecologically significant responses of microbial communities to contaminants, and their potential impact on higher trophic levels in aquatic food webs. While various studies have investigated the impacts of pesticides on the structure and diversity of aquatic microbial communities (*e. g*. Debenest et al. 2010, Staley et al. 2015), information is still needed to understand how pesticides affect ecosystem functions supported by microorganisms.

Leenhardt et al. (2022) proposed a framework using a set of clearly-defined core categories of ecosystem functions and services supporting the identification of effectively or potentially threatened function. For instance, microbial autotrophs are involved in ecosystem functions production of organic matter (OM) and the provision and maintenance of biodiversity. They also contribute to gas regulation given their photosynthetic character and to the cycling of nutrients. Microbial heterotrophs play a pivotal role in the regulation of carbon and nutrient cycles, gas exchanges with the atmosphere, propagule dispersion, and pollutant mitigation in the aquatic ecosystem (Leenhardt et al. 2022). Both the autotrophic and heterotrophic components are involved in the provision and maintenance of biotic interactions within the microbial community and through the aquatic food web. The ecosystem function categories described in Leenhardt et al. (2022) are based on the works by De Groot et al. (2002) and Pettorelli et al. (2018) with some modifications. Ecosystem functions supported by microbial communities can be directly measured or estimated using a set of proxies which simplifies the methodological approach.

In this context, we performed a systematic review of pesticides' effects on the ecosystem functions associated with aquatic microbial communities over the last twenty years. The main objectives of this review were i) to characterise the main functions assessed and how they are impacted by pesticide exposure and ii) to identify overlooked microbial functions and future research perspectives to better consider the effects of pesticides on the ecosystem functions supported by microbial communities.

2. Systematic review of AME studies addressing ecosystem functions: Methodology

We retrieved all Web of Science publications (including original research articles, reviews and book chapters) published in English between 2000 and 2020 to gather existing knowledge on pesticides' effects towards the diversity and functions of aquatic microbial communities. The literature search was performed on the Title and Author keywords, by combining requests (detailed in Supplementary Information: SI1) related to aquatic ecosystems (query #1) and pesticide exposure (query #2), with the targeted microbial communities (query #3 for the autotrophic component or query #4 for the heterotrophic component).

From these combinations of queries, we harvested a limited number of relevant references for the autotrophic component of microbial communities. More specifically, 258 references were retrieved,

80 of which 61% were finally discarded (Figure 1). A total of 517 references were retrieved for the
81 heterotrophic component. Among these 517 references, only 18% were retained (Figure 1). After
82 clean-up, a complete list of 182 references were conserved for data analysis, where records related
83 to autotrophic and heterotrophic components of microbial communities were balanced (Figure 1, see
84 reference list in SI2) . We observed a very low overlap of references between queries made for
85 autotrophic or heterotrophic components (7%) highlighting the fact that studies cover ecosystem
86 functions supported by one or the other specific component of aquatic microbial communities.
87 Overall, studies distinguish pesticide effects between autotrophic and heterotrophic components of
88 microbial communities are few, although several functions can be ensured by both (i. e. respiration
89 and nutrient uptake, among others…).

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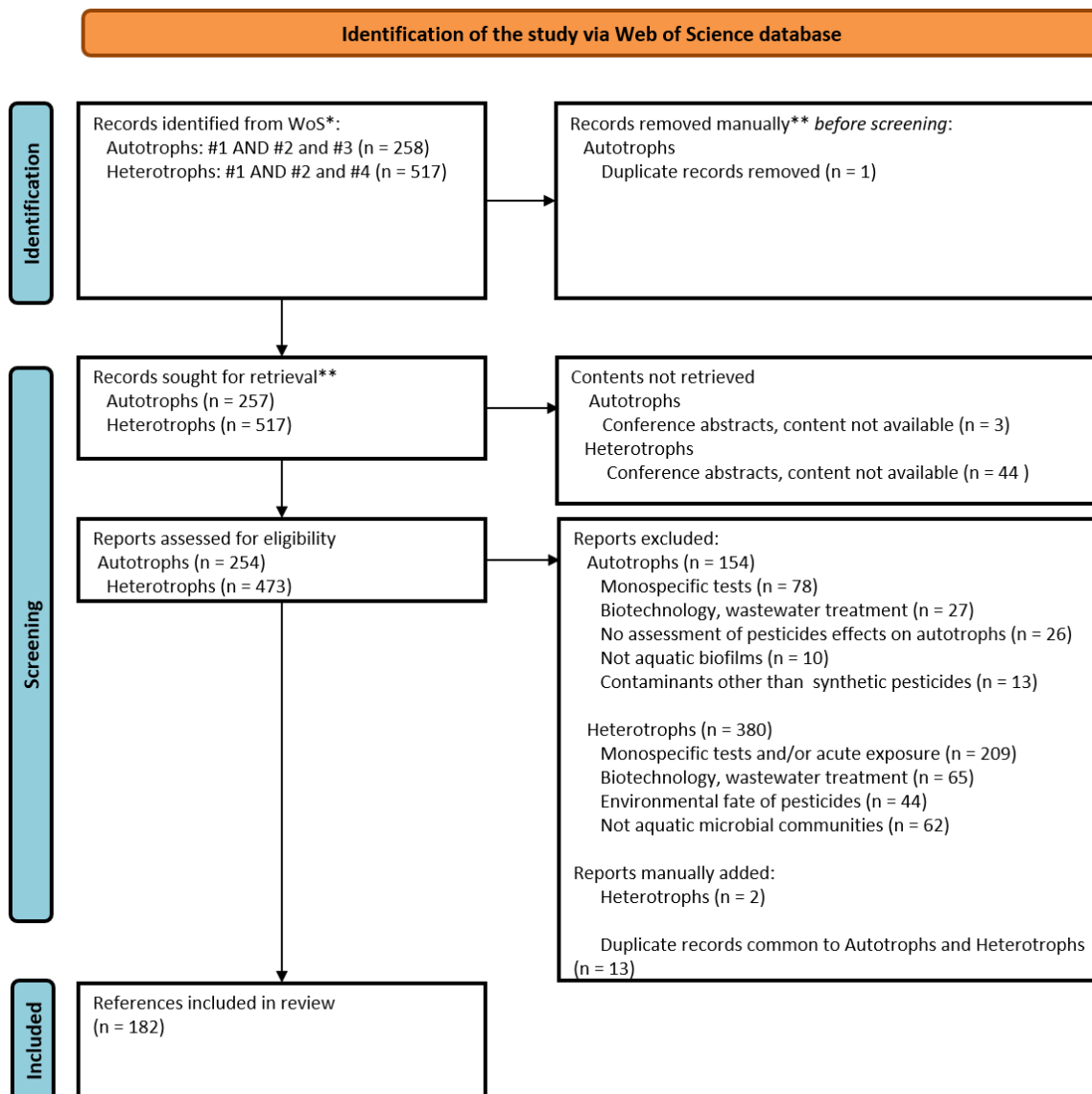
91 Figure 1. PRISMA 2020 flow diagram for the systematic review (Page et al. 2021). Detailed search
92 requests are available in SI1 (*). No automation tools were used (**).

93 **3. Results and discussion**

94 *3.1. Communities targeted and experimental conditions*

95 The first outcome of our literature review was that most studies (72%) analysed the impacts of
96 pesticides on communities living in biofilms rather than in planktonic habitats (Table 1). AME covers
97 studies in all types of aquatic ecosystems; however, much more studies have been conducted in
98 freshwaters (85% of the records) than in marine or transitional ecosystems (Table 1; see also Zhao et
99 al. 2022). This trend observed in the literature review could be due to the dilution effect of pollutants
100 in marine waters compared to freshwaters (Pesce et al. 2021; Leenhardt et al. 2022). Accordingly,
101 most studies in the marine environment were conducted in the coastal zone close to sources of
102 pollution. Among freshwater ecosystems, 56% of the studies dealt with lotic environments (streams
103 and rivers) followed by studies in lentic environments (ponds, lakes and reservoirs, 28%). A
104 consistently lower percentage of studies conducted in lentic environments was observed for
105 heterotrophic components compared to autotrophic parts of microbial communities (Table 1).

106 This literature review focusing on AME studies performed at the microbial community and ecosystem
107 scales highlights that both experimental (nanocosms, microcosms and mesocosms, *sensu* Newman
108 and Clements 2008) and field approaches (i. e. watershed) are used, but with some preferences:
109 Table 1 indicates that 69% of AME studies were conducted at the community level in microcosms (i. e.
110 test tubes, laboratory jars, small aquaria, etc...) followed by field studies and mesocosms (e. g.
111 artificial flumes, ponds, enclosures) approaches. Comparatively, the use of microcosm approaches
112 compared to field and mesocosm approaches is much more imbalanced when studying heterotrophic
113 than autotrophic parts of the microbial communities. The strong reproducibility and increasing
114 standardisation (e. g. OECD 2006) of micro- and mesocosm approaches make them a highly
115 complementary tool for discerning the effects of pesticide in the natural environment. Despite
116 efforts made by the AME researchers' community to better link laboratory to field results, recurring
117 difficulties persist in filling the gaps (Vignati et al. 2007; Artigas et al. 2012a).

Number of records	Autotrophic part	Heterotrophic part
Habitat		
-Benthic (biofilm)	60	73
-Planktonic/pelagic	28	13
-Both habitats	7	5
Environment		
-Lotic	50	54
-Lentic	40	14
-Coastal/estuarine	5	23

Experimental approach		
-Microcosms/nanocosms	45	80
-Mesocosms	18	4
-Field	32	7

Table 1: Habitat, type of aquatic environment and experimental approach implemented in AME studies between 2000 and 2020 for autotrophic components (n = 95) and heterotrophic components of aquatic microbial communities (n = 91).

Forty-five pesticides were considered in the references analysed, which is low compared to the large number of pesticides used and present in aquatic environments (e. g. Sharma et al. 2019). As expected, the mode of action of the pesticides tested was in line with the main microbial component studied (Figure 2). Articles targeting autotrophic components of aquatic microbial communities mostly addressed the impact of herbicides (60% of studies), in particular photosynthesis inhibitors (atrazine: 18 records, followed by diuron: 13 records, and isoproturon: 7 records) and the broad-spectrum herbicide glyphosate (phosphonoglycine, 18 occurrences). The highest diversity in herbicide molecules belongs to the chemical families of chloroacetamides (7 molecules dominated by (S-)metolachlor: 4 records over a total of 11), triazines (26 records for 5 compounds, dominated by atrazine) and ureas (diuron, isoproturon and chlortoluron totalizing 21 occurrences). In the case of heterotrophic components, the effects of fungicides have been addressed predominantly (61% of studies), followed by herbicides and to a lesser extent insecticides. However, a similar diversity of fungicide and herbicide molecules were tested. Tebuconazole (22 records, triazole) and chlorothalonil (20 records, chloronitrile) in the case of fungicides, and glyphosate (17 records, phosphonoglycine) in the case of herbicides, were the most studied molecules when dealing with pesticides effects on the heterotrophic component of aquatic microbial communities.
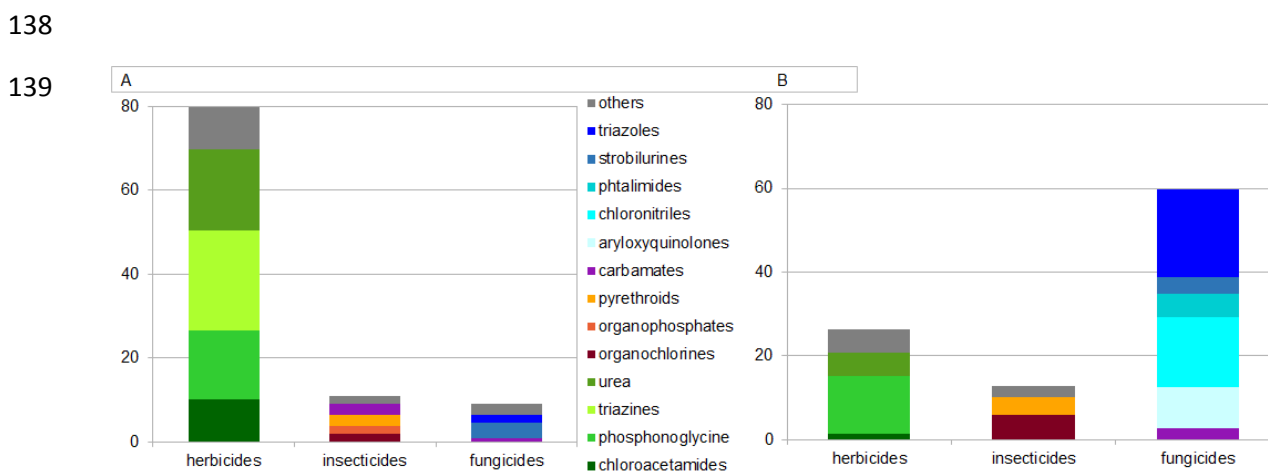
Figure 2: Chemical families of the 3 main classes of pesticides (bars = counts, 1 family may contain several molecules) tested against aquatic microbial communities under controlled conditions, excluding field and micro/mesocosm studies where complex environmental mixtures of pesticides were present. Note that several studies include 2 or more pesticide molecules, sometimes

144   considering mixture effects. Autotrophic component (plot A) n = 108 occurrences, 45 chemicals.
145   Heterotrophic component (plot B) n = 120 occurrences, 34 chemicals.

146

147   *3.2. General overview of functional endpoints affected by pesticide exposure*

148   Aquatic microbial communities, including autotrophs and heterotrophs, support a large number of
149   ecosystem functions (Leenhardt et al. 2022) that can be impacted by pesticide exposure. While most
150   studies in the literature have addressed pesticide effects on the biomass and diversity of microbial
151   communities, only half attempted to assess pesticide effects on other ecosystem functions.
152   Moreover, a large number of publications mention functional endpoints as highly sensitive to
153   pesticide exposure, often providing earlier or exacerbated responses compared to structural
154   endpoints (such as taxonomic composition; e. g. Sabater et al. 2007).

155   The framework of clearly-defined core categories of ecosystem functions and services by Leenhardt
156   et al. (2022) helped us to identify which functions are effectively or potentially threatened by
157   pesticides (Table 2). Most studies on the heterotrophic component of aquatic microbial communities
158   have examined the effect of pesticides on functions related to the provision and maintenance of
159   biodiversity and biotic interactions (Figure 3). The effects of pesticides on heterotrophs' biodiversity
160   and interactions have been much more studied for bacteria than for fungal communities (65% and
161   33%, respectively), and the effects on protozoan communities remain almost unexplored (2%).
162   Besides, the processes of OM decomposition (36%) and respiration (14%) were the most examined
163   when assessing pesticide effects on carbon and nutrient cycles and gas regulation functions. To a
164   lesser extent, hyphomycete sporulation and bacterial production were employed to determine
165   pesticide effects on propagule dispersion and OM production, respectively. In the autotrophic
166   component, pesticides' impact on biodiversity was also widely studied (73%). Then, primary
167   productivity (58%) and photosynthesis (22%) impairment were the most examined processes when
168   assessing pesticide effects on the production of OM and gas regulation functions, which is consistent
169   with the large number of studies focusing on the impact of molecules with an herbicidal mode of
170   action (inhibitors of photosystems I and II, Figure 2). Few references explored the interactions with
171   higher trophic levels (8%), targeting biofilm grazers. Indeed, the large majority of the effects
172   assessed refer to the direct impacts of pesticides towards specific components of aquatic
173   communities, herbicides vs. autotrophic components and fungicides vs. heterotrophic components
174   (Figure 2). A limited number of articles report the indirect impact of pesticides in microbial
175   communities due to alterations in interactions between microorganisms (competition, facilitation; e.
176   g. Sura et al. 2012, Artigas et al. 2014) or with higher trophic levels (changes in grazing pressure; e. g.
177   Rohr & Crumrine 2005, Bundschuh et al. 2011, Neury-Ormanni et al. 2020).

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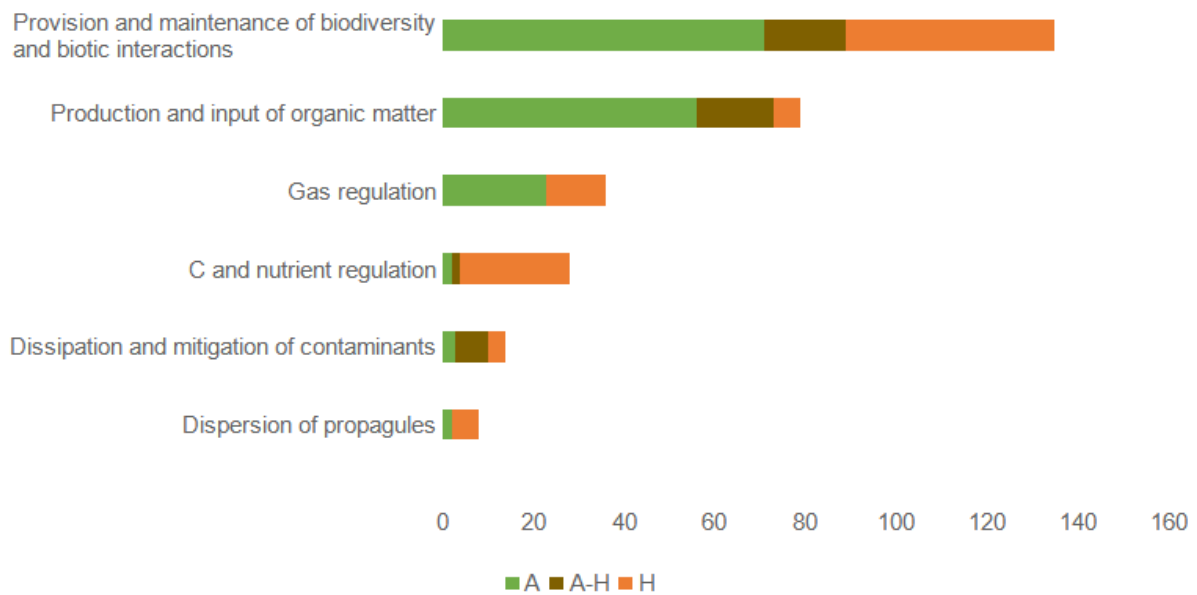
Figure 3. Number of AME studies examining several ecosystem functions between 2000 and 2020 for autotrophic (A), heterotrophic (H), or both (A-H) components of aquatic microbial communities (n=168). Note that several functions may be considered in a single record.

The choice of the functional endpoints studied is generally adapted to the temporality of exposure to pesticides: physiological descriptors allow highlighting short-term toxicity, while impacts on biomass, or changes in taxonomic composition, generally operate for a longer duration of exposure (Sabater et al. 2007). Even if the choice of the experimental approach (microcosm, mesocosm, *in situ;* Table 1) has an influence on the results and conclusions that can be drawn, it did not determine the choice of functional parameters in the studies analysed here. For instance, molecular-level endpoints were used in field experiments (e. g. *nif* genes, Sun et al. 2012) and ecosystem-level functional endpoints were used in microcosm experiments (e. g. primary production, OM decomposition, Gardeström et al. 2016). Nevertheless, experiments under controlled conditions (i. e. microcosms) tended to use molecular-level endpoints permitting a mechanistic understanding of pesticide effects on microbial processes. Finally, several studies used multiple endpoint approaches targeting the same microbial function (i. e. from the gene expression to the enzyme activity, e. g. Su et al. 2019). Despite various studies showing a clear correlation between responses of multiple endpoints to pesticides (e. g. Artigas et al. 2012b), others showed more mixed results (e. g. Pesce et al. 2016). For instance, the structural and functional endpoints of leaf-associated microbial assemblages can be decoupled when exposed to contaminants, suggesting that contaminants effects may be misinterpreted when solely based on structural endpoints (Feckler and Bundschuh 2020).

200 Table 2. Main ecosystem functions, processes and endpoints measured in microbial community ecotoxicology studies during between 2000 and 2020.
201 Functions can be ensured by autotrophs (A), heterotrophs (H) or both (A and H, when one is dominant the other is mentioned in brackets). Examples of
202 references (non-exhaustive) using the methods are provided, together with the main observed effects of pesticide exposure towards the corresponding
203 ecosystem functions.

Ecosystem function	Ecosystem process	Main community targeted (A, H, A-H)	Endpoints used	Analyses and techniques	References using the method (examples)	Observed impact of pesticide exposure
Provision and maintenance of biodiversity and biotic interactions in aquatic ecosystems	Population/community dynamics	A-H	Growth rates	Cell increase over time using microscope counts	Moreira-Santos et al. (2005), Hayashi et al. (2011), Proia et al. (2011), Larras et al. (2016)	Decrease
				Cell increase over time using flow cytometry	de la Broise & Stachowski-Haberkorn (2012), Bricheux et al. (2013), Artigas et al. (2017)	
			Cell densities, live/dead ratio	Cell densities measurements using microscope counts	Perez et al. (2007), Debenest et al. (2009), Vera et al. (2010), Proia et al. (2011), Kim-Tiam et al. (2014, 2015), Lozano et al. (2018)	
	Biodiversity	A	Taxonomic composition	Taxonomic analysis using microscope identification	Bérard et al. (2003), Morin et al. (2009, 2010), Magnusson et al. (2012), Roubeix et al. (2012), Kim-Tiam et al. (2014, 2015), Feckler et al. (2018b), Smedbol et al. (2018), Bighiu et al. (2020)	Selection of pollution tolerant taxa/groups, to the detriment of sensitive taxa/groups
		A		Pigment composition analysis using HPLC	Pinckney et al. (2002), Dorigo et al. (2007), Magnusson et al. (2012)	

		A		Pigment composition analysis using fluorimetry techniques	Morin et al. (2010), Kim-Tiam et al. (2015), Polst et al. (2018)	
		A-H		Community structure analysis using DGGE/TGGE	Dorigo et al. (2007, 2010), Tien et al. (2013), Paule et al. (2015a)	
		A-H		Community diversity analysis using high-throughput sequencing	Dimitrov et al. (2014), Lu et al. (2019)	
	Competition/facilitation	A-H	Diversity/co-occurrence analyses	Species density analyses using microscope counts	Proia et al. (2011)	Targeted groups outcompeted by non-target components
	Trophic interactions	A	Production of essential fatty acids	Analysis of fatty acid profiles using gas chromatography	Filimonova et al. (2016, 2018), Demailly et al. (2019), Zhang et al. (2020)	Decrease
		A-H	Trophic interactions through food web approaches	Multiple trophic levels design using density or diversity dynamics analyses	Rohr & Crumrine (2005), Chang et al. (2011), Cothran et al. (2011), Zubrod et al. (2011), Neury-Ormanni et al. (2020)	Weak indirect effects (herbicides), reduced top-down impacts (insecticides)
Production and input of organic matter in aquatic ecosystems	Primary productivity	A	Gross and net primary production	O2 production using respiration chambers	Murdoch et al. (2013)	Decrease (herbicides), no effect or increase (fungicides, insecticides)
				Inorganic 14C incorporation using radioisotopic techniques	Vera et al. (2010), Tlili et al. (2011), Villeneuve et al. (2011)	
			Chlorophyll-a concentration	Chlorophyll-a concentration analysis using HPLC	Gustavson et al. (2003), Magnusson et al. (2012), Feckler et al. (2018b), Bighiu et al. (2020)	

				Chlorophyll-a concentration analysis using spectrophotometric techniques	Morin et al. (2010), Murdoch et al. (2013), Abhelo et al. (2016), King et al. (2016), Lozano et al. (2018), Smedbol et al. (2018)	
				Chlorophyll-a concentration analysis using fluorimetry techniques	Bérard et al. (2003), Schmitt-Jansen & Altenburger (2005), Pesce et al. (2010)	
	Biomass production	A-H	Periphyton production over time	Determination of Dry weight, Ash-Free Dry Mass, Particulate C, over time	Dorigo et al. (2010), King et al. (2016), Feckler et al. (2018b)	Decrease at elevated concentrations of exposure
		H	Fungal production over time	Analysis of ergosterol concentration over time using HPLC	Artigas et al. (2012), Dimitrov et al. (2014), Gardeström et al. (2016)	Decrease in fungal production (fungicides) and increase in bacterial production
		H	Bacterial production over time	Leucine incorporation over time using radioisotopic techniques	Widenfalk et al. (2004), Widenfalk et al. (2008), Milenkovski et al. (2010)	
				Thymidine incorporation over time using radioisotopic techniques	Pesce et al. (2006), Pesce et al. (2008), Sura et al. (2012), Artigas et al. (2014)	
Gas regulation	C cycle: Photosynthesis	A	Photosynthetic activity	Inorganic 14C assimilation using radioisotopic techniques	Gustavson et al. (2003), Schaffer & Sebetich (2004), Perez et al. (2007)	Decrease (herbicides), sometimes recovering over

				Photosystem II efficiency using PAM techniques	Schmitt-Jansen & Altenburger (2005), Ricart et al. (2009), Magnusson et al. (2012, 2013), Smedbol et al. (2018)	time after low-dose exposure
C cycle: Respiration	H (A)	Dissolved oxygen consumption rate	Oxygen consumption analysis using respiration chambers	Kreutzweiser et al. (2007)		Decrease (fungicides), neutral or stimulatory effects of herbicides and insecticides
			CO2 production analysis using 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC) reduction	Pesce et al. (2018), Pesce et al. (2006)		
		Carbon dioxide production rate	CO2 production analysis using Gas chromatography, Substrate-induced respiration (MicroResp)	Chen et al. (2019), Artigas et al. (2014), Mbanaso et al. (2014), Widenfalk et al. (2004)		
N cycle: Denitrification	H	N fluxes	Denitrification flux analysis using the acetylene inhibition method	Milenkovski et al. (2010)		No effect or negative for fungicides
			N flux analysis using 15N isotopic techniques	Widenfalk et al. (2004), Su et al. (2019), Chen et al (2019)		

			Denitrification-involved enzymes rates (nitrate reductase, nitrite reductase, nitric oxide reductase, nitrous oxide reductase)	Enzyme activity measurements using dyes (ex. Viologen) reduction bioassay	Su et al. (2019), Chen et al (2019)	
	S cycle: Sulfate reduction	H	Sulfate reduction rate	Sulfate reduction analyses using turbidimetric method (sulfate) and copper spectrophotometric method (sulfide)	Garcia-Cruz et al. (2010)	Decrease (herbicides and metabolites)
Carbon and nutrient regulation in aquatic ecosystems	Organic carbon and nutrient recycling	A-H	N-uptake (NO3)	N uptake analysis using Tracer addition techniques	Mulholland et al. (2004)	Transient decay then recovery
			N-uptake (NH4)	N uptake analysis using ammonium decay measurements	Murdoch et al. (2013)	
			P-uptake	P uptake analysis using soluble reactive phosphorus measurements	Proia et al. (2011)	
		H	Decomposition rates	Analysis of mass loss of leaf litter, cotton or wood strips	Artigas et al. (2012), Dimitrov et al. (2014), Kreutzweiser et al. (2007), Brosed et al. (2016), Rossi et al. (2018)	decrease (or no effect)

			Extracellular enzymatic activity rates (cellulolytic, ligninolytic, peptidases, phosphatases)	Enzyme activity measurements using fluorescent methylumbelliferone-substrate analogues	Ricart et al. (2009), Artigas et al. (2012b), Donnadieu et al. (2016), Rossi et al. (2018)	variable effect depending on pesticide and enzyme activity type
			Metabolic richness and diversity	C-substrates utilisation using Biolog Ecoplates	Campbell et al. (2003), Foley et al. (2008), Sura et al. (2012), Paule et al. (2015a,b), Pringault et al. (2016), Lv et al. (2017), Kumar et al. (2020)	
Dissipation and mitigation of contaminants	Transformation and mineralisation of contaminants	H (A)	Dissipation rates	Contaminants dissipation analysis using mass balance calculations	Thomas & Hand (2011), Tien et al. (2013), Paule et al. (2015a), Carles et al. (2017)	Increase
		H	Mineralization rates	Contaminantes mineralisation analysis using radiorespirometry techniques	Pesce et al (2013), Pesce et al. (2010)	
	Removal of contaminants	A-H	Organic pesticide bioaccumulation	Contaminants accumulation analysis using GC-MS analysis	Lawrence et al. (2001), Zhao et al. (2015), Qiu et al. (2017), Rooney et al. (2020)	
				HPLC-MS, UPLC-ToF analysis	Chaumet et al. (2019)	
Dispersion of propagules in aquatic ecosystems	Sporulation	H	Hyphomycetes sporulation rates	Sporulation rates determination using conidia counts and identification under the microscope	Flores et al. (2014), Donnadieu et al. (2016), Brosed et al. (2016), Pimentao et al. (2020)	Decrease

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209 *3.3. Impacts of pesticides on ecosystem functions ensured by autotroph-dominated microbial*
210 *communities*

211

212 *Provision and maintenance of biodiversity*

213 In the autotrophic component of microbial communities, the impairment of biodiversity by
214 herbicides is one of the impacts most frequently studied, thanks to a large range of available
215 methods. Classic microscopic methods allowing algal identification were often complemented by
216 other techniques, from rough estimates of algal groups based on pigment profiles to genetic
217 approaches (electrophoresis being recently supplied by high-throughput sequencing). Several studies
218 demonstrated changes in the algal composition and diversity of phytoplankton and periphyton
219 exposed to herbicides, exposure generally leading to the selection of pollution-tolerant algal groups
220 to the detriment of sensitive individuals. In the presence of the broad-spectrum herbicide
221 glyphosate, studies observed higher vulnerability of chlorophytes and diatoms to herbicides, and
222 increasing amounts of cyanobacteria (Pérez et al. 2007, Smebold et al. 2018). More contrasted
223 responses of algal groups (diatoms in particular) were shown with other herbicides, such as the
224 widely studied photosynthesis inhibitor diuron. Community changes induced by diuron as well as
225 other pesticides were assessed by Dorigo et al. (2007, 2009, 2010) in the Morcille watershed (South-
226 East France). Their work highlighted the increase of diatoms and cyanobacteria to the detriment of
227 green algae along the contamination gradient, based on pigment signatures (HPLC measurements).
228 Villeneuve et al. (2011) assessed the effects of long-term exposure of biofilms to diuron and the
229 fungicide azoxystrobin by microscopic counts; they found that diatoms were more tolerant to
230 pollution than cyanophytes and chlorophytes. Using fluorescence measurements, on the contrary,
231 higher amounts of green algae were detected downstream of pesticide inputs (Kim-Tiam et al. 2014)
232 in the same watershed, reflecting interannual variability or divergences between microscopy, HPLC
233 and fluorimetry results. The application of the herbicide simetryn on phytoplankton communities
234 caused a decrease in the abundance of chlorophyceae (*Chlamydomonas* sp.) but did not affect
235 significantly diatoms (Chang et al. 2011), confirming the ability of the latter to maintain under
236 exposure to photosynthesis inhibitors.

237 Note that the vast majority of the effects assessed refer to direct impacts of herbicides on
238 community composition; however, some studies report indirect effects of pesticides, related to
239 changes in the relationships between organisms in the community. An overall decrease in
240 biodiversity was also demonstrated with fungicides. For instance, Lu et al. (2019) exposed
241 phytoplankton to azoxystrobin; they concluded that competition relationships were altered under
242 pesticide exposure, favouring cyanobacterial dominance at the detriment of chlorophytes. Abelho et
243 al. (2016) found that the fungicide pyrimethanil decreased fungal biomass in biofilms, altering the
244 relative abundances of periphytic fungi and algae. On the contrary, exposure to the insecticide
245 pyridaben enhanced the diversity of planktonic algal communities (Rand et al. 2001), compared to
246 reference conditions. This could be due to decreased top-down control or medium enrichment by
247 dead insect bodies (Knapp et al. 2005).

248 The impacts of pesticide exposure on biodiversity considering species-specific sensitivities of the
249 autotrophic component of microbial communities mostly targeted diatoms, probably as a
250 consequence of their tolerance to various pesticides (see above, or Rimet & Bouchez 2011; Bricheux
251 et al. 2013) and of their wide use as environmental bioindicators (Coste et al. 2009; Morin et al.

252 2016; Water Framework Directive 2000/60/EC). As for algal groups, a selection of tolerant species
253 was often observed, together with a general decrease in species diversity. In several field studies
254 attempting to characterize the specific sensitivity of taxa to pesticides, diatom diversity metrics
255 highlighted eutrophication more than pesticide exposure (Morin et al. 2009, 2010; Roubeix et al.
256 2012; Wood et al. 2019; Bighiu et al. 2020), as both pollutions often occur simultaneously in
257 agricultural watersheds. This may explain why diatom species able to colonize
258 eutrophic/hypereutrophic environments, such as *Nitzschia palea* or *Planothidium frequentissimum*,
259 were considered more tolerant to herbicides (e. g. Debenest et al. 2010). To disentangle the nutrient
260 from the pesticide effects, works were performed under laboratory-controlled conditions (e. g.
261 Villeneuve et al. 2011; Tien et al. 2013; Bayona et al. 2014; Wood et al. 2017) and generally
262 highlighted a pesticide-induced selection of tolerant taxa and an overall decrease in diversity. Other
263 confounding factors were found to mask the deleterious impacts of pesticides on the taxonomic
264 structure of periphyton, such as light availability (Feckler et al. 2018b). Another approach combining
265 diatom traits, taxonomic metrics and diatomic indices in multimetric models was proposed by Larras
266 et al. (2017). They showed that pesticides selected for motile diatom species, highlighting the
267 promising use of approaches based on traits or ecological life forms (Rimet & Bouchez 2011, Marcel
268 et al. 2013) for assessing toxic-induced species selection in diatoms.
269

270 *Provision and maintenance of biotic interactions*

271 As primary producers, photosynthetic microorganisms are essentially at the base of aquatic food
272 webs. The cascading effects of pesticides along the trophic chain have been examined mainly under
273 controlled conditions.

274 Direct deleterious effects of herbicides on the autotrophic component of microbial communities
275 were sometimes shown to decrease the biomass of grazers, with effects that are difficult to detect in
276 most cases. For instance, Rohr & Crumrine (2005) demonstrated that the decrease in periphyton
277 biomass after atrazine exposure caused a decrease in the biomass and reproduction of snails and
278 tadpoles. In contrast, Muñoz et al. (2001) found, in a simple periphyton-snails design, that the
279 biomass of periphyton decreased with the addition of atrazine, while that of grazers was not affected
280 by the herbicide. Chang et al. (2011) assessed how another triazine herbicide, simetryn, impacted a
281 planktonic microbial food web composed of phytoplankton, bacteria, heterotrophic nanoflagellates,
282 and ciliates. The highest concentration caused a decrease in the biomass of phytoplankton, especially
283 in green algae, but weak direct and indirect effects on zooplankton. They suggested that other
284 microorganisms unimpacted by the herbicide could constitute alternative food for zooplankton. In
285 complement, Neury-Ormanni et al. (2020) suggested that a poor microalgae quality subsequent to
286 diuron exposure (e. g. decreased content in essential fatty acids, see below), could trigger
287 invertebrates to graze higher amounts of contaminated microalgae, to compensate for the decrease
288 in nutrient supply (also see "Dissipation and mitigation of contaminants"). The impacts of glyphosate
289 on phytoplankton were also shown to be altered in the presence of golden mussels (*Limnoperna*
290 *fortunei*), which addition modified nutrient content in the water column and selected for
291 phytoplankton species (De Stefano et al. 2018; Gattás et al. 2018). Such results contrast those of
292 Iummato et al. (2017) who found no differences in periphyton exposed or not to glyphosate in the
293 presence of *L. fortunei* suggesting that the mussels attenuated the effects of glyphosate by
294 contributing to its dissipation, or of López-Doval et al. (2010) who did not observe any significant
295 interactions between diuron, biofilms and grazers (freshwater snails *Physella acuta*).

On the other side, the exposure of herbivorous grazers to insecticides can modulate the responses of phototrophic communities: the interactions are often marginal but can be notable in the case of insecticides (Cothran et al. 2011; Hua & Relyea 2012; Neury-Ormanni et al. 2020) where there are few direct effects on primary producers but reduced top-down pressure resulting from toxicity on grazers, indirectly favouring the development of algae.

At the molecular level, some studies looked at the synthesis of proteins, fatty acids or carbohydrates, and the incorporation of nutrients (cited for example in Debenest et al. 2010). Such biomolecules are key in the diet of higher organisms and the provision of energy to the consumers; the impairment of the synthesis of fatty acids is recently given increasing attention with the development of targeted and non-targeted metabolomics (Zhang et al. 2020). The supply of essential fatty acids (omega 3 and omega 6) along the trophic chain is mainly ensured by microalgae, in particular diatoms (Gonçalves et al. 2016). The consequences of pesticide exposure on their nutritional value are crucial for ecosystem functioning, as the impacts on the basis of the food web could have severe repercussions for higher trophic levels. Herbicide-induced alterations in fatty acid profiles were noticeable in decreasing the proportion of highly unsaturated fatty acids including the eicosapentaenoic acid (omega 3) of monospecific cultures of diatoms (Filimonova et al. 2016, 2018; Demailly et al. 2019). Conversely, in a metabolomic study, Zhang et al. (2020) observed a significant upregulation of some omega 3 and omega 6 fatty acids (e. g. linolenic acid, linoleic acid and arachidonic acid) in a phytoplanktonic community exposed to the fungicide azoxystrobin, likely a consequence of reduced competition in the community.

Finally, certain photosynthetic organisms can be sources of toxic molecules, and the presence of pesticides may favour them directly. For example, Lürling & Roessink (2006) demonstrated that the cyanobacteria *Microcystis aeruginosa* outcompeted chlorophytes (*Scenedesmus obliquus*) in the presence of the herbicide metribuzin. In the same way, Khadra et al. (2018) showed that glyphosate exposure was likely to induce blooms of the potentially toxic genus *Anabaena* in lacustrine biofilms.

Although many authors mentioned the impairment of food provision/energy supply as a possible consequence of the toxic effects measured, those studies rarely addressed directly the impact of pesticide exposure on the transfer of OM and energy to higher trophic levels.

Production and supply of organic matter

To assess the impacts of herbicides, autotrophic biomass is often mentioned in freshwater aquatic microbial communities, together with photosynthesis (see "Gas regulation"). Literature reviews (Pesce et al. 2011, or Debenest et al. 2010 focusing on diatoms) highlighted the negative effects of herbicides on the algal component of freshwater biofilms, using biomass, microscope countings, or pigment content. Besides rough estimates such as dry weight or ash-free dry mass, concentrations of chlorophyll-*a* are often used as an estimate of the biomass of photosynthetic microorganisms in phytoplankton and periphyton. Considering freshwater microalgae, the recent review by Vonk & Kraak (2020) confirmed that a high number of studies dealt with the ecotoxicity of pesticides targeting photosynthesis (triazines, ureas) and other herbicides (e. g. chloroacetamides) based on global endpoints such as abundance, biomass or growth descriptors. For most of these compounds, median EC_{50}s was below the mg/L range, suggesting that toxicity should occur at environmental concentrations with visible impacts on OM production by autotrophs.

338   In most studies, long-term exposure (weeks to months) to herbicides impaired the function of
339   production and supply of OM by autotrophic components of microbial communities. These
340   herbicides reduced the chlorophyll-*a* concentration (Murdock et al. 2013; Rohr & Crumrine 2005),
341   growth dynamics and primary production (measured as 14C incorporation) of periphytic
342   communities (Villeneuve et al. 2011). In several field studies where exposure to pesticides is complex
343   (mixtures of molecules) at concentrations in the range of ng-μg/L, negative impacts on periphytic
344   biomass were observed (e. g. Morin et al. 2010; Kim-Tiam et al. 2014). The diversity of works
345   assessing the growth responses of periphyton exposed to one particular pesticide shows that
346   exposure concentration and the chemical composition of pesticides matter. Dealing with glyphosate
347   for instance, Smedbol et al. (2018) used environmentally realistic concentrations of the herbicide
348   (from μg to mg of active ingredient (a.i.)/L) and demonstrated a decrease in chlorophyll-*a* and
349   carotenoid concentrations of phytoplankton communities from the lowest concentration of
350   exposure. Opposite results were found by Pérez et al. (2007) using higher concentrations of
351   glyphosate: the primary production of phytoplankton exposed to milligrams a.i./L of glyphosate
352   roughly doubled compared to control conditions, which could be related to increased phosphorus
353   concentrations in the medium supplied by glyphosate additions (Saxton et al. 2011, but see Carles &
354   Artigas 2020). Such an increase in biomass (assessed as dry weight or chlorophyll-*a* concentration)
355   was confirmed recently by Vera & Trinelli (2021) after a 7-day exposure of periphyton to
356   concentrations around the mg a.i./L of glyphosate. In other cases, glyphosate exposure had no effect
357   on chlorophyll a content but only impacted their structural composition (see "Provision and
358   maintenance of biodiversity"), highlighting the capacity of biofilms to regulate and maintain a
359   constant level of algal biomass (Pesce et al. 2009; Bricheux et al. 2013).

360   Contrastingly, studies using pesticides other than herbicides generally did not observe a decrease in
361   microalgal biomass (e. g. Artigas et al. 2014, for the fungicide tebuconazole), sometimes even an
362   increase. For instance, Abhelo et al. (2016) found increased microalgal biomass compared to fungal
363   biomass, under exposure to the fungicide pyrimethanil. Rohr & Crumrine (2005) found that exposure
364   to the insecticide endosulfan did not affect the biomass of periphytic algae, estimated by chlorophyll-
365   *a*. Rand et al. (2001) showed no effects on periphyton communities but highlighted a positive
366   correlation of phytoplankton abundance with exposure to the insecticide-miticide pyridaben. In such
367   studies, it is important to highlight that a "positive" impact of pesticides on growth may rather derive
368   from decreased competition between microbial components or reduced grazing (i. e. top-down
369   pressure) (Rand et al. 2001), as described in "Provision and maintenance of biotic interactions".

370   Albeit the effects of pesticides on this function are widely studied, most of these works have
371   estimated effects via proxies under controlled conditions rather than measuring effects in the field.

372

373   *Gas regulation*

374   Some studies also looked at the consequences of pesticide exposure on gross primary production
375   and respiration in autotrophic biofilms, generally by recording dissolved oxygen in incubation
376   chambers, under light and dark conditions respectively. Respiration was, in general, unimpacted by
377   pesticide exposure, while some studies highlighted a decrease in gross primary production after
378   contamination. Murdoch et al. (2013) measured these functions in wetland periphyton after an
379   agricultural runoff. They found interactive effects with total phosphorus causing a respiration
380   decrease, and atrazine mitigating the negative relationship between respiration and phosphorus.

381 Using laboratory chambers, they found no impact of atrazine on the respiration of periphyton on the
382 third day of exposure but a decrease in gross primary production at the highest concentration of
383 exposure. Villeneuve et al. (2011) also observed a decrease in the primary production of biofilms
384 exposed to a mixture of pesticides, even if no decrease was detected in algal density. Lozano et al.
385 (2018) found transient effects of herbicides (2,4-D, glyphosate and their mixture) on the respiration
386 of freshwater phytoplankton. Immediately following application, the herbicides caused a decrease in
387 the respiration rate of microalgal communities in mesocosms, but after 1 day the effect was no
388 longer detected. This may explain why, in river biofilms exposed to glyphosate, Artigas et al. (2020)
389 found no significant impact on net primary production nor respiration rates analysed from 4 days and
390 later on.

391 Despite the photosynthetic character of microalgae, the influence of pesticides on gas regulation in
392 microalgal communities remains poorly studied in freshwaters. The general decline in research
393 dedicated to primary productivity in periphyton over the last three decades was recently highlighted
394 by Zhao et al. (2022). At a global scale, the part of freshwaters in the total amount of water on planet
395 Earth is very low (1%) compared to oceans (> 95%) where the contribution of phytoplankton to the
396 world's primary production is paramount (Field et al. 1998). Moreover, with the development of
397 PAM (Pulse Amplitude Modulated) fluorimetry techniques over the last 20 years, chlorophyll-*a*
398 fluorescence parameter (e. g. photosynthetic efficiency) are increasingly used as endpoints of
399 pesticide toxicity (Table 2; this proxy representing 76% of photosynthesis assessments in the corpus).
400 Chlorophyll-*a* fluorescence bioassays have been applied successfully to assess the ecotoxicity of a
401 wide range of contaminants (e. g. metals, herbicides, petrochemicals), as such tests are rapid, non-
402 invasive and non-destructive (Ralph et al. 2007). Most of the studies retrieved using PAM techniques
403 highlighted photosynthesis impairment with herbicide exposure; however, such measurements stray
404 from the primary assessment of the ecosystem function of gas regulation related to photosynthesis.

405

406 *Nutrient regulation*

407 The impacts of pesticides on the function of regulation of nutrient cycles relative to freshwater
408 microalgae are mentioned, for example in the review by Debenest et al. (2010). Some herbicides can
409 reduce the absorption of nutrients (nitrates, nitrites, phosphorus and silica, in particular). In their
410 review of the environmental consequences of herbicide impacts on cyanobacteria, Brêda-Alves et al.
411 (2021) showed that increasing concentrations of several herbicides in aquatic environments impair
412 atmospheric nitrogen fixation by cyanobacteria, as a result of photosynthesis inhibition. Such a
413 decrease is likely to impact the overall aquatic nitrogen cycle. However, in their study addressing the
414 effects of atrazine on laboratory periphyton, Murdoch et al. (2013) observed an early, sharp
415 reduction of NH_4^+ uptake rates (75 to 84%) after 3 days of exposure, followed by a recovery of
416 control rates after 1 week. According to these observations, the effects of pesticide exposure on
417 nutrient cycles may be transient. Concerning P cycle, Proia et al. (2011) did not observe any change in
418 phosphorus uptake by periphyton exposed for 2 weeks to diuron.

419

420 *Dissipation and mitigation of contaminants*

421 Living in biofilms can buffer microorganisms from variations in the external environment, including
422 exposure to pesticides. Besides, autotrophic biofilms can bioaccumulate, and thus partly remove,
423 organic pesticides from water. In their recent review, Bonnineau et al. (2021) demonstrated that

424 freshwater periphyton can accumulate organic pesticides present in the water column with bio-
425 uptake efficiencies varying according to their hydrophobicity, estimated by their partition coefficient
426 between octanol and water (log Kow). Besides, Vonk & Kraak (2020) reported that uptake rates are
427 influenced by biological characteristics (cell size and lipid composition of the organisms).
428 Bioconcentration factors are calculated as the ratio of pesticide concentration in the biofilm with
429 respect to its concentration in surrounding water, and values over 1 indicate bioconcentration. In
430 Canadian wetlands (Rondeau Bay, Ontario), Rooney et al. (2020) found that periphyton
431 bioconcentrates a large variety of pesticides, with bioconcentration factors ranging from 12 for the
432 herbicide dicamba up to 6864 for the fungicide boscalid. Removal of pesticides by microalgae from
433 their environment was also shown to occur in phytoplankton. In lakes, high bioconcentration factors
434 for organochlorine pesticides were detected in phytoplankton (Zhao et al. 2015; Qiu et al. 2017), with
435 a high affinity of pesticides for diatoms and cryptophytes. However, bioconcentration of pesticides in
436 primary producers may expose other aquatic biota via consumption (Qiu et al. 2017; Rooney et al.
437 2020), and therefore have deleterious consequences on biotic interactions ("Provision and
438 maintenance of biotic interactions").

439 The capacity of microalgae to detoxify organic contaminants has been shown using several
440 wastewater-treatment microalgal technologies (e. g. Pazos et al. 2016; Sutherland & Ralph 2019);
441 data in natural aquatic ecosystems are scarce. Paule et al. (2015) compared the removal rates of
442 alachlor by wastewater and river biofilms showing that, even 10 times lower for natural biofilms, the
443 rates of disappearance of the pesticides reached 5-10 µg alachlor removed daily per gram of biofilm
444 dry weight. Such transformation capacities of natural periphytic biofilms can contribute to the self-
445 purification of rivers downstream pesticide inputs. In their review on cyanoremediation, Kumar and
446 Singh (2017) highlighted the biodegradation capabilities of cyanobacteria, with some genera
447 common in freshwaters (e. g. *Anabaena, Microcystis, Nostoc, Spirulina*) able to degrade various
448 pesticides. Tien et al. (2013) assessed the capacity of freshwater autotrophic biofilms to biodegrade
449 carbamate pesticides (methomyl, carbaryl, carbofuran). Although toxic effects of the pesticides were
450 observed on communities of diatoms and bacteria (See "Provision and maintenance of biodiversity"),
451 they found that tolerant diatoms and bacteria were potential degraders of the three carbamate
452 pesticides tested alone. However, their ability to break down the pesticides was impaired when
453 mixtures were tested, suggesting a decrease in rivers' bioremediation ability under complex
454 contamination conditions. In another study, Lawrence et al. (2001) proved that atrazine and diclofop
455 methyl were mineralized by river biofilms to CO_2. In most studies, observed biotransformation
456 reactions mostly corresponded to substitution-type reactions catalyzed by central metabolic
457 enzymes ubiquitously found in bacteria. Although correlations with any autotrophic component were
458 not assessed, similar enzymatic machinery exists in microalgae (Sheng et al. 2022). Thomas & Hand
459 (2011) showed that, in environmental systems, the presence of algae increased the rate of
460 degradation of several pesticides compared to sediment alone.

461

462 Summarizing the main data available, pesticide exposure often impacted the following ecosystem
463 functions supported by autotrophs in microbial communities:

464 -Provision and maintenance of biodiversity and trophic interactions, highlighting the selection of
465 tolerant microalgae (taxa or groups) and a decrease in diversity, while cascading effects were

466 generally weak or hardly observable. However, the current development of dietary tracers (e. g. fatty
467 acids) is a promising approach to shape future research;

468 -Production and supply of OM, showing a general trend of decreasing microalgal biomass with
469 herbicide exposure, while fungicides and insecticides did not significantly impair autotrophic biomass
470 as the result of decreased competition or predation;

471 -Gas regulation, through the reduction of gross primary production and impairment of
472 photosynthesis.

473

474 *3.4. Impacts of pesticides on ecosystem functions ensured by heterotroph-dominated microbial*
475 *communities*

476 *Provision and maintenance of biodiversity and biotic interactions*

477 Microbial heterotrophs play a pivotal role in aquatic food webs as both organic matter (OM)
478 decomposers and OM suppliers to higher trophic levels (Gessner and Chauvet 1994). Little is known
479 about cascading effects of pesticides exposure on aquatic food webs, especially for the heterotrophic
480 component of microbial communities (e. g. Zubrod et al. 2011). Most of these experiments were
481 conducted in microcosms and revealed both "top-down" and "bottom-up" effects in natural
482 microbial communities resulting from pesticide exposure. For instance, exposure to low deltamethrin
483 concentrations increased drastically arthropod mortality followed by a sudden increase of activity in
484 bacterial and algal communities in the water column (Knapp et al. 2005). This sequential response
485 can be explained by an apparent sudden release of nutrients following the death of the arthropods,
486 which triggered a series of responses in the microbial loop. Pesticides can also reduce OM
487 consumption by several aquatic invertebrate species (e. g. Zubrod et al. 2015), often driven by
488 changes in the OM-associated microbial community (e. g. Feckler et al. 2016). For instance, the
489 exposure to a mixture of five current-use fungicides (azoxystrobin, carbendazim, cyprodinil,
490 quinoxyfen, tebuconazole) reduced leaf consumption by gammarids, probably due to the reduction
491 in the richness of fungal species (ca. 40%) which contribute to reducing the nutritional quality of the
492 leaves (Zubrod et al. 2015). Diet-related effects impairing the functioning of the shredder *Asellus*
493 *aquaticus* resulting from epoxiconazole exposure were partially explained by lowered microbial
494 biomasses and altered composition of fatty acids associated with the leaf material (Feckler et al.
495 2016).

496 Few studies have attempted to investigate the effect of pesticides in microbial heterotrophs
497 interactions (i. e. fungus-fungus, bacteria-fungi, bacteria-protozoa, etc...) in the aquatic environment
498 (see review by Proia et al. 2012). For instance, the fungicide tebuconazole significantly reduced the
499 fungal biomass and increased that of bacteria in leaves and stream sediments in a microcosm
500 experiment (Donnadieu et al. 2016). The authors suggested that the increase in bacterial biomass
501 could be explained by (i) reduced resource competition between fungi and bacteria when fungi are
502 stressed by the fungicide and/or (ii) enhanced supply of nutrients released by the killed fungi and
503 further used by bacteria to grow. Indeed, this second hypothesis was also observed in soil microbial
504 communities after tebuconazole application (Cycoń et al. 2006). Within a biofilm, the effects of
505 grazing by protozoa on the structure of the bacterial community have been demonstrated (e. g. Matz
506 and Kjelleberg 2005). In this sense, the study of Friberg-Jensen et al. (2003) shows that reduced

507   grazer control from crustaceans due to cypermethrin exposure resulted in the proliferation of
508   rotifers, protozoans, bacteria, and algae from plankton and periphyton.
509

510   *Production and supply of organic matter*

511   Few studies have investigated the effect of pesticides on OM production by aquatic microbial
512   heterotrophs (e. g. Artigas et al. 2014, Pesce et al. 2006). In these studies, bacterial production was
513   analysed with two different radioisotopic techniques: leucine and thymidine incorporation. The
514   effect of herbicides alone or in mixtures, as well as fungicides alone or in mixtures, were tested on
515   bacterial production in periphytic, planktonic and sediment microbial communities. The literature
516   review highlighted that pesticides have little impact on bacterial production, with the vast majority of
517   responses being neutral or transient.

518   For instance, bacterial production measured in plankton and sediment microbial communities from
519   European lakes showed a transient decrease following exposure to the fungicides tebuconazole
520   (Artigas et al. 2014) and captan (Widenfalk et al. 2004), as well as exposure to the insecticides
521   deltamethrin and pirimicarb (Widenfalk et al. 2004). Exposure to environmentally realistic
522   concentrations of the herbicides diuron and glyphosate did not influence bacterial production in
523   stream periphyton communities (Pesce et al. 2006; 2009).

524   Studies in microcosms made it possible to assess the impact of pesticide cocktails on bacterial
525   production in aquatic microbial communities. Overall, no effects of cocktails of fungicides
526   (Milenkovski et al. 2010), herbicides (Sura et al. 2015; Pringault et al. 2016) or mixtures of herbicides,
527   fungicides and insecticides molecules (Widenfalk et al. 2008) were observed on bacterial production.
528   Field studies concluded also that pesticide contamination gradients observed in rivers and wetlands
529   from Europe weakly affected the bacterial production in aquatic microbial communities (Pesce et al.
530   2008 and Sura et al. 2012, respectively).

531   Fungal production measured as acetate incorporation into ergosterol or biomass accrual estimates
532   has not been employed in aquatic microbial ecotoxicology studies during the last 20 years. Instead,
533   fungal production in the form of conidia (sporulation) has been widely used as an endpoint to assess
534   pesticide effects on aquatic hyphomycetes communities. We decided to address pesticide effects on
535   aquatic hyphomycetes sporulation in the section below "Dispersion of propagules".

536
537   *Gas regulation*

538   The assessment of the impact of pesticides on microbial gas exchanges with the atmosphere has
539   focused mainly on the carbon and nitrogen cycles (e. g. Widenfalk et al. 2004, Chen et al. 2019).
540   Studies assessing the impact of pesticides on the sulfur- and methane gases are rare in literature (e. g.
541   Garcia-Cruz et al. 2010). The study of endpoints related to respiration and denitrification processes is
542   the most used in aquatic microbial ecotoxicology. Respiration and denitrification appeared less
543   responsive to pesticides in aquatic environments with an equal number of studies showing negative
544   or neutral effects. Respiration rates in sediment microbial communities from a reservoir system in
545   China decreased as a function of a gradient of fungicide (chlorothalonil) and insecticide
546   (propetamphos) concentrations (Su et al. 2019, Chen et al. 2019). Similar results were observed in
547   sediment microbial communities from an estuary zone in England (Garcia-Ortega et al. 2011). The
548   exposure history of microbial communities to pesticide contamination appears to determine the
549   response of respiration to the fungicide tebuconazole (Artigas et al. 2014). For instance, periphyton

550 respiration rates decreased after exposure to environmental concentrations of tebuconazole in a site
551 less contaminated by pesticides than in a site more contaminated by pesticides. Substrate-induced
552 respiration (SIR) is one of the most frequently used techniques to estimate respiration rates in
553 microbial communities (e. g. Tlili et al. 2011). It is important to note that SIR measurements include
554 the contribution of both autotrophs and heterotrophs in the respiration process, and therefore
555 caution must be taken when distinguishing pesticide effects between components of the microbial
556 community. The accumulation of high concentrations (mg/L) of metabolites from the herbicide 2,4-D
557 (4-chlorophenol, 2-chlorophenol and phenol) can inhibit the respiration rates from biofilms
558 dominated by sulfur-reducing bacteria (Garcia-Cruz et al. 2010). High concentrations of the herbicide
559 glyphosate can display neutral or stimulating effects on the respiration rates of microbial
560 communities from streams (Artigas et al. 2020) and ditch systems (Mbanaso et al. 2014), respectively.
561 Neutral effects of the herbicide diuron and the insecticide imidacloprid were observed in
562 communities from epilithon (Pesce et al. 2006) and associated with decomposing leaf litter
563 (Kreutzweiser et al. 2007) in streams, respectively. Laboratory studies (Widenfalk et al. 2004) and
564 field studies (Pesce et al. 2008) have coincided in describing the neutral effects of pesticide cocktails
565 (composed of fungicides, herbicides and insecticides) on the respiration of heterotrophic microbial
566 communities, as previously mentioned for autotrophs (see 3.3).

567 The denitrification rates and enzymatic activities involved in the denitrification activity in sediments
568 from the Three Gorges reservoir (China) decreased according to the increase in chlorothalonil
569 concentrations (Chen et al. 2019; Su et al. 2019). However, the expression of genes involved in the
570 denitrification activity (e. g. *nirK*, *nirS*, *narG*...) were not sensitive to chlorothalonil. These results from
571 these experiments show that multi-marker approaches (gene expression versus enzyme activity
572 measurement) often used in aquatic microbial ecotoxicology may result in contradictory conclusions.

573
574 *Carbon and nutrient regulation*

575 Pesticides impact has been extensively studied in the decomposition process of particulate OM
576 (mostly leaf litter) by heterotrophic components of microbial communities. This process has been
577 mostly examined in stream and river ecosystems where the availability of allochthonous OM prevails
578 over that of autochthonous origin (e. g. Zubrod et al. 2011, Rasmussen et al. 2012, Rossi et al. 2018).
579 Two main functional endpoints are used to assess pesticide effects on OM decomposition: the mass
580 loss of plant materials (e. g. Brosed et al. 2016) or the measurement of extracellular enzymatic
581 activities involved in the decomposition of plant materials (e. g. Artigas et al. 2012b). Other studies
582 have addressed the impact of pesticides on the metabolic diversity (i. e. community-level
583 physiological profiles; Foley et al. 2008, Sura et al. 2012) or the expression of certain functional genes
584 (i. e. coding carbohydrates and lignin degradation processes) of heterotrophic microbial communities
585 for the utilisation of a variety of organic carbon or nutrient sources; however these studies are few in
586 the literature (Chen et al. 2019, Su et al. 2019, Lu et al. 2020).

587 Studies show that the decomposition of leaf litter by microbial heterotrophs is sensitive to pesticides,
588 and only a few studies show neutral effects. Negative effects on litter decomposition were observed
589 when fungicides are applied, probably due to their direct effect on aquatic hyphomycete
590 communities responsible for leaf decomposition (Artigas et al. 2012b; Fernandez et al. 2015;
591 Gardestrom et al. 2016; Dawoud et al. 2017; Feckler et al. 2018b; Rossi et al. 2018). This is the case
592 for environmental concentrations of azoxystrobin and tebuconazole which were able to decrease
593 fungal biomass, litter decomposition rates and cellulolytic enzyme activities (β-glucosidase, β-

594   xylosidase ou cellobiohydrolase) (Gardestrom et al. 2016; Dawoud et al. 2017; Artigas et al. 2012b).
595   However, the extent of the effect of fungicides on microbial OM decomposition depends on: i) the
596   type of aquatic ecosystem studied (Dimitrov et al. 2014; Donnadieu et al. 2016; Pesce et al. 2016), ii)
597   the exposure history of communities to contamination (Gardestrom et al. 2016), iii) the OM quality
598   (Artigas et al. 2012b), and iv) the availability of dissolved nutrients in stream water (Rossi et al. 2018).
599   Tebuconazole can also increase certain ligninolytic enzyme activities during leaf decomposition, not
600   because of the degradation of leaf polymers, but probably because of the detoxification and/or
601   biodegradation mechanisms employed by heterotrophic components of microbial communities
602   (Rossi et al. 2018; Artigas et al. 2017). The impact of herbicides (glyphosate, Kennedy et al., 2012)
603   and insecticides (imidacloprid; Kreutzweiser et al. 2007, 2008) have been shown to weakly affect
604   microbial litter decomposition, even if slight changes in fungal biomass accumulation and/or
605   communities' composition (fungal and bacterial) are observed.

606   Very few studies have assessed the effects of pesticides in the decomposer-detritivore system, to
607   determine how pesticide effects on decomposers (bacteria and fungi) may affect detritivores
608   (macroinvertebrate shredders) based on their trophic relationships (i. e. Zubrod et al. 2011). For
609   instance, the fungicide tebuconazole has been observed to modify the structure of leaf-associated
610   microbial communities and alter the consumption of leaves by shredder macroinvertebrates (Zubrod
611   et al. 2011). Cornejo et al. (2021) showed that the decomposition of leaves in tropical streams was
612   especially reduced when fungicides and insecticides are combined (chlorpyrifos + chlorothalonil),
613   suggesting that each pesticide operates differently on microbial and macroinvertebrate communities
614   and their effects are additive. Field studies assessing pesticide impact on microbial decomposition of
615   particulate OM are also scarce, and conclusions are often contradictory. For instance, the study by
616   Brosed et al. (2016) showed that the breakdown rate of alder leaves strongly decreased along a
617   pesticide concentration gradient in 12 French streams due to effects on invertebrate's
618   decomposition but not on microbial decomposition. Piscart et al. (2011) observed that the response
619   of microbes to litter decomposition may not be sensitive enough for assessing the global effect of
620   seasonal agricultural practices. In contrast, the study of Fernandez et al. (2015) observed a strong
621   relationship between the fungicide toxicity gradient and the decrease in microbial litter
622   decomposition in 17 German streams. Rasmussen et al. (2012) observed also that microbial litter
623   decomposition was reduced by a factor of two to four in agricultural streams compared to forested
624   streams, and suggested that microbial decomposition activity responded more strongly to pesticide
625   toxicity rather than to eutrophication. Cause and effect relationships between pesticides and
626   microbial litter decomposition in the field are difficult to establish, and authors often employ "toxic
627   unit" calculations based on toxicity data (EC_{50}) from a few model organisms (i. e. microalgae) to
628   correlate with microbial decomposers activity. In view of these approaches, fungal and bacterial
629   toxicity data are needed (see Maltby et al. 2009, Ittner et al. 2018) to properly assess the impact of
630   pesticides on microbial litter decomposition in field studies.

631   The impact of pesticides has been assessed on the diversity of carbon substrates utilisation by
632   bacterial communities from rivers (Foley et al. 2008), wetlands (Sura et al. 2012; Lv et al. 2017) and
633   coastal ecosystems (Pringault et al. 2016). Two herbicide cocktails, one composed of 2,4-D, MCPA,
634   dicamba, clopyralid, dichlorprop, mecoprop, bromoxynil, glyphosate, and the other composed of
635   diuron, di-isopropyl-atrazine, 3,4-dichlorophenylurea, alachlor and linuron, altered the metabolic
636   diversity of biofilm communities from two wetlands in Manitoba, Canada (Sura et al. 2012) and
637   strongly reduced the carbohydrate utilisation (and increased that of amino acids and polymers) of
638   bacterioplankton communities from a coastal bay in Tunisia (Pringault et al. 2016). Exposure to

639 environmental concentrations of the herbicide acetochlor increased the overall quantity, but not
640 diversity, of carbon substrates utilisation by freshwater bacterial communities from US streams
641 (Foley et al. 2008). Instead, the fungicides imazalil and tebuconazole did not affect the metabolic
642 diversity of sediment bacterial communities from constructed wetlands in Denmark (Lv et al. 2017).
643 Few ecotoxicology studies have shown the impact of pesticides on metabolic diversity, which could
644 be explained by certain methodological constraints when measuring the use of carbon substrates in
645 heterotrophic microbial communities (see Preston-Mafham et al. 2002). For instance, plating may
646 induce a strong selection pressure in microbial species which would not reflect the actual
647 metabolism of microbial communities in the field. Moreover, the tetrazolium dye immediately
648 introduces some bias since not all bacteria nor fungi can reduce it, hence the plates do not
649 necessarily give a complete picture of the microbial metabolism.

650

651 *Dissipation and mitigation of contaminants and wastes*

652 The study of microbial communities' capacities for the mitigation of pesticides has been largely
653 studied in literature, mostly in the soils compared to aquatic environments (see Fenner et al. 2013).
654 However, very few studies have investigated how exposure to pesticides may affect the microbial
655 communities' capacity to biodegrade further pesticide molecules. The limited literature on this
656 subject shows that aquatic microbial communities already exposed to chronic pesticide
657 contamination in the field tend to display a higher potential to degrade certain pesticide molecules (*i.
658 e.* nicosulfuron; Carles et al. 2017). Nevertheless, this observation is pesticide-dependent since
659 exposure history to pesticides was overridden by phosphorus limitation in glyphosate degradation by
660 stream biofilms (Carles et al. 2019). Co-metabolism of pesticides has often been observed in
661 heterotrophic microbial communities (Liu et al. 2000). This is explained by a large number of sources
662 of naturally occurring OM available to the microbial community, a condition greatly favouring the co-
663 metabolic degradation of pesticides and the production of potentially recalcitrant metabolites
664 (Fenner et al. 2013). For instance, the biodegradation kinetics of nicosulfuron by *Plectosphaerella*
665 *cucumerina* AR1 (isolated from decomposing leaves in streams) depended on glucose concentration,
666 with a maximum specific degradation rate at 1 g/L in glucose (Carles et al. 2018). Another relevant
667 factor described by Fenner et al. (2013) is the pesticide threshold concentrations below which
668 microbial biodegradation is slowed down: this is the case of low biodegradation in groundwater
669 environments where pesticide concentrations are low and moleculess can persist for longer periods.

670

671 *Dispersion of propagules*

672 The production of aquatic fungi in AME studies is mostly analysed through the counting and
673 taxonomic identification of conidia produced by aquatic hyphomycete communities (Gessner and
674 Chauvet 1994). The sporulation rates of hyphomycetes are quite sensitive to pesticides, especially
675 fungicides, with half of the studies showing a negative impact on this functional endpoint (e. g.
676 Zubrod et al. 2011; Dimitrov et al. 2014; Pimentao et al. 2020) and the other half showing neutral
677 and/or positive effects (e. g. Dawoud et al. 2017). A microcosm study showed that repeated
678 applications of the fungicide azoxystrobin decreased the sporulation rates of the hyphomycete
679 community colonizing black alder leaves, and this decrease was more marked in fungal assemblages
680 from uncontaminated forest streams compared to those from pesticide-contaminated sites
681 (Gardestrom et al. 2016). The effect of the fungicide tebuconazole on hyphomycetes sporulation

682   varied between studies, with some clear negative effects on one side (Zubrod et al. 2011; Dimitrov et
683   al. 2014; Pimentao et al. 2020) but also with some examples of stimulatory effects (Donnadieu et al.
684   2016; Dawoud et al., 2017). A possible explanation for these contradictory results may be the
685   concentration of tebuconazole used and/or the different community species composition between
686   experiments.

687   In contrast to the previous studies, Dawoud et al. (2017) observed a positive effect of high
688   tebuconazole concentrations on the sporulation of hyphomycetes which became neutral when
689   communities were contaminated by both tebuconazole and the insecticide lindane. The sporulation
690   of hyphomycetes colonizing alder leaves from a pristine stream was insensitive to the pharmaceutical
691   antifungal terbinafine (Pimentao et al. 2020). Pesticide contamination gradients in the Garonne
692   watershed (France) did not affect hyphomycete sporulation rates (Brosed et al. 2016), neither the
693   pesticide mixture composed of the fungicide imazalil and the insecticide diazinon (Flores et al. 2014).

694

695   Summarizing the main data available, pesticide exposure often impacted the following ecosystem
696   functions supported by heterotrophs in microbial communities:

697   -Carbon and nutrient regulation functions are impaired by pesticides. A reduction in litter
698   decomposition rates and a decrease in fungal biomass, is observed in microcosm studies assessing
699   fungicides toxicity. Bacterial communities are less sensitive to pesticides and often supplant fungi
700   altered by pesticides. However, these trends are less consistent in field studies where contamination
701   gradients contain not only pesticides but also nutrients, metals, pharmaceuticals, etc.

702   -Propagules dispersal (sporulation rates) in aquatic hyphomycete communities is one of the most
703   sensitive parameters to pesticide exposure in heterotrophic microbial communities, whereas
704   extracellular enzymatic activities often show transitory responses to pesticides exposure.

705   -Biotic interactions may change between decomposers and detritivores depending on the pesticide
706   applied. Despite changes in biotic interactions, the decomposition function of OM tends to remain
707   unchanged.

708

709   *3.5. Under-researched functions in AME studies*

710   A considerable number of ecosystem functions supported by microbial communities remain under-
711   researched. For instance, the gas regulation function provided by aquatic microbial communities has
712   been mostly focused on major (carbon and nitrogen) rather than on minor (i. e. sulfur) element
713   cycles. Few studies have investigated the effect of pesticides on anaerobic respiratory processes in
714   aquatic environments. For instance, OM decomposition experiments are often conducted in aerobic
715   habitats (i. e. water column or benthic surface) rather than in anaerobic habitats (i. e. hyporheic zone)
716   where OM may also accumulate. For instance, Bollinger et al. (2022) observed a twofold higher
717   fungicide effect in the hyporheic zone on microbial leaf litter decomposition compared to the benthic
718   zone. Garcia-Cruz et al. (2010) agreed on the presence of chlorophenols (i. e. 2,4-
719   dichlorophenoxyacetic acid (2,4D)) in environments where sulfate-reducing bacteria are present and
720   cause toxicity and inhibition on sulfate respiration.

721   Similar to gas cycles, most AME studies focus on pesticide effects on macro-nutrient cycles (i. e. C

722   and N cycles) rather than on micro-nutrient cycles (i. e. potassium, iron, calcium, ...). Moreover, the

723   impact of pesticides on macro-nutrient cycles is often assessed via proxies (i. e. gene expression,

724   potential enzymatic activities) rather than using tracer addition approaches (i. e. NO_3-^{15}N, Mulholland

725   et al. 2004) which are more integrative of microbial nutrient uptake capacities. Few studies have

726   examined the effect of pesticides on the phosphorus uptake capacity of periphyton by measuring the

727   temporal decay of soluble reactive phosphorus (SRP) (e. g. the herbicide diuron, Proia et al. 2011).

728   Further, AME studies could also integrate pesticides in carbon and nutrient cycling of contaminated

729   environments since pesticides can represent a nutrient resource for aquatic microorganisms (e. g.

730   Carles et al. 2019).

731   Overall, there is a lack of studies assessing the impacts of pesticides on the functions shared by

732   autotrophic and heterotrophic microorganisms (11% of the references explicitly consider the

733   functions as ensured by both components, see Figure 3). For instance, gas exchange (i. e. respiration),

734   OM production, nutrient regulation (i. e. nutrient uptake or enzymatic activities) or pesticide

735   dissipation functions measured at the community level reflect the sum of metabolic processes

736   carried out by both autotrophs and heterotrophs in the microbial community. Further, studies at the

737   ecosystem scale should consider the sum of metabolisms measured in the different habitats of the

738   ecosystem. Indeed, a range of studies shows the importance of considering the metabolism from

739   both planktonic and littoral habitats in lakes when assessing carbon fluxes at the ecosystem scale

740   (Vadeboncoeur et al. 2001; Vesterinen et al. 2017).

741   Finally, the protozoan community has been largely overlooked in AME studies despite contributing to

742   gas and nutrient regulation, production and supply of OM, and to the provision and maintenance of

743   biotic interactions in aquatic ecosystems. While some studies have attempted to assess pesticide

744   effect on ciliate and flagellate populations densities (e. g. Chang et al. 2011; Neury-Ormanni et al.

745   2016; Lu et al. 2020), studies assessing effects on ecosystem functions ensured by protozoans are

746   missing in the literature. Lu et al. (2020) observed that abundances of eukaryotic microbes increased

747   in the whole zooplankton community of lake Taihu (China), especially rotifers, which is in accordance

748   with the phenomenon that rotifers increase their hatchling proportion under exposure to glyphosate

749   (Gutierrez et al. 2017).

4. Research perspectives for AME studies based on ecosystem functions

This section highlights some of the research aspects missing in the literature review, that deserve further investigations in order to enhance fundamental understanding in AME and improve risk assessment in regard to the use of pesticides.

First of all, determining whether the impact of contaminants is transferred along the biological continuum (from the genes to the ecosystem) will enable us to identify molecules with high ecological impact. The effects of contaminants may occur at all levels of biological organization, from molecular to ecosystem-level responses (Clements 2000). However, extrapolating cause-effect relationships observed at the microbial population level to the community level is problematic because of diverse biotic interactions (including both intra- and interspecific) and complex gradients of environmental factors influencing the propagation of pesticide effects. The influence of biotic interactions in leaf-associated microbial communities exposed to fungicides has been studied (Artigas et al. 2017). The response of the extracellular laccase activity to the fungicide tebuconazole was different when assessing the entire community response or the responses of their individual components. Laccase activity of individual populations of bacteria and/or fungi was more sensitive to the fungicide rather than that of the entire microbial community. The authors suggested that this different response was explained by a range of biotic (i. e. species diversity and interactions) and abiotic (i. e. community architecture, molecules exchange) factors avoiding the propagation of pesticide effects from the population level to the community level. Similarly, the tolerance to diuron was different when examining responses at the periphyton community level or at the algal population level of an agricultural stream. While pollution-induced community tolerance to diuron was observed for periphytic communities in the downstream site (more contaminated) compared to those from the upstream site (less contaminated), certain diatom strains (i. e. *Encyonema neomesianum*) collected from the downstream site were more sensitive than those collected from the upstream site (Roubeix et al. 2012). The authors from this study suggested that these differences were partly explained by the co-occurrence *in situ* of copper and diuron contamination gradients, *E. neomesianum* strains from downstream being more tolerant to copper. Extrapolation of pesticide effects between levels of biological organisation is thus tricky given the environmental context of multi-contamination. Beyond these studies, identifying "keystone species'' within microbial communities and testing the effect of pesticides on these specific populations could be a research strategy to better address the propagation of pesticide effects at the community level or even the entire ecosystem.

Second, we observed a very low number of references (7%) considering explicitly pesticide effects on the autotrophic and heterotrophic components at the same time, permitting to have a more integrated view of the overall response of the aquatic microbial community to contaminants. Assessing simultaneously functions ensured by both autotrophs and heterotrophs could be a way to better integrate the impacts of pesticides on the entire ecosystem functioning. Such a research challenge could be overcome by the miniaturization of tests permitting to process a large number of samples. Tests in microplates are already used for the assessment of pesticide effects on microbial photosynthesis (e. g. Gardia-Parège, Kim-Tiam et al. 2022), or carbon substrate use (e. g. Foley et al. 2008, Sura et al. 2012, Pringault et al. 2016, Lv et al. 2017). Moreover, studies permitting to have a more integrated response of the ecosystem (i. e. trophic chains such as decomposers-detritivores-carnivores or primary producers-herbivores-carnivores) are rare in the literature. Some studies on leaf litter decomposition observed that the contamination by pesticides does not impair microbial-mediated decomposition but often decreases invertebrate-mediated decomposition (Piscart et al.

795 2011; Brosed et al. 2016; Rossi et al. 2019; Jabiol et al. 2022). Despite such a top-down effect of
796 pesticides in the decomposer-detritivore system, other studies have shown bottom-up effects in the
797 producer-consumer system explained by pesticides' effect on food resources quality (rarefaction of
798 species rich in omega-3 and 6 fatty acids) to consumers (see sections "Provision and maintenance of
799 biotic interactions" in 3.3 and 3.4). Microbial communities can bioaccumulate pesticides (see sections
800 "Dissipation and mitigation of contaminants" in 3.3 and 3.4) and contribute to their transfer through
801 the trophic chain. In turn, consumers can also adapt their diet as a function of the content (nutritious
802 quality and amount of contaminants) by avoiding some unsuitable food sources or diversifying them
803 (Neury-Ormanni et al. 2020, Bundschuh et al. 2011). This topic certainly deserves more research by
804 comparing different types of exposure conditions, cocktails of molecules (including metabolites), and
805 trophic interactions.

806 Third, examining the species selection and their physiological and metabolic adaptations in
807 environments chronically contaminated by pesticides will permit us to determine the recovery
808 potential of these communities to a non-contaminated status. Both autotrophic and heterotrophic
809 components of aquatic microbial communities have shown strong functional adaptability of
810 microbial communities chronically exposed to pesticides, even if this contradicts the literature survey
811 by Allison and Martigny (2008) in which microbial communities are sensitive to disturbance and often
812 do not rapidly recover to their original state after chronic exposure. Blanck (2002) described this
813 adaptation process based on profound structural changes in microbial communities' composition, i. e.
814 the elimination of sensitive species followed by the development of pesticide-tolerant species in the
815 microbial community, in his so-called "pollution-induced community tolerance" concept. However,
816 structural changes in pesticide-polluted communities are not always accompanied by functional
817 changes and this is because tolerant species can compensate for the loss of sensitive competitors
818 and maintain ecosystem functions. Unfortunately, the knowledge of the presence or absence of
819 sensitive and tolerant species offers little information on their functional capacities, since strong
820 functional redundancy exists in microbial communities (see Tlili et al. 2016). The study of Feckler et al.
821 (2018a) showed comparatively minor adverse effects of fungicides or even stimulation of ecosystem
822 functions in microbial communities previously exposed to agricultural contamination. Similar results
823 were observed by Pesce et al. (2010) with the effect of the herbicide diuron in periphyton
824 communities chronically exposed to pesticide contamination. Research efforts are still needed to
825 better establish the functional traits characteristic of microbial species (Bier et al. 2015) and their
826 responses to pesticides. Beyond that, the study of genetic mutations in microorganisms resulting
827 from selection pressure due to exposure to pesticides is poorly investigated in aquatic microbial
828 communities, compared to soils (e. g. Pileggi et al. 2020). Understanding the versatility of microbial
829 species to adapt to pesticides and their functional role will certainly help to understand the
830 functioning of microbial communities in environments chronically contaminated by pesticides.

831 Fourth, unravelling "omics" multi-functional responses of microbial communities to pesticides will
832 allow us to detect, through non-targeted analyses, unsuspected functional responses to pesticides.
833 The literature review of Ebner (2021) found only 648 studies addressing "omics" and "ecotoxicology"
834 in the last twenty years (2000-2020) which is a relatively low score. This review also concludes that
835 transcriptomics is the most frequently applied method (43%), followed by proteomics (30%),
836 metabolomics (13%) and finally, multi-omics (combination of two or multiple omics methods, 13%).
837 We still identify a gap in the potential use of omics data to explain multiple functions in microbial
838 communities. Johnson et al. (2015) pioneered in suggesting an "association mining approach"
839 between the rate constants of observed biotransformation reactions and meta-omics data as an

840 untargeted approach to generate hypotheses about potential causal linkages between enzymes and
841 pesticides biotransformation. Recently, Achermann et al. (2020) used association mining to
842 demonstrate quantitative correlations between metatranscriptomic data and micropollutant
843 biotransformation in activated sludge. We consider that extrapolation of association mining between
844 chemical and metatranscriptomic profiling to enzymes likely involved in catalysing reactions related
845 to important ecosystem functions, under natural conditions, would help in bridging the gap between
846 -omics data potential and the wide diversity of functions ensured by microbial communities.

847

5. Concluding remarks

849 As highlighted by this literature review of the last 20 years of AME research, ecosystem functions
850 ensured by aquatic microbial communities are threatened by pesticide contamination. It is important
851 to remark that the assessment of pesticide effects on microbial functions is based on a limited
852 number of pesticide molecules (mainly, herbicides for autotrophs and fungicides for heterotrophs)
853 which does not allow for a complete picture of the multiple contaminations affecting aquatic
854 microbial communities. Surprisingly, AME works that focused on autrotrophs mostly studied aspects
855 related to the functions of provision and maintenance of biodiversity and biotic interactions, while
856 AME studies on heterotrophs mostly examine functions related to carbon and nutrient regulation.
857 Integrative ecotoxicological risk assessment from microbial communities to ecosystem functions calls
858 for more complex experiments and requires exchanges between more or less distant fields of
859 research. We believe that incorporating measurements of function, as often done in ecology
860 approaches, would put forward ecosystemic consequences of pesticide exposure and allow to some
861 extent the quantification of the impacts of pesticide pollution on ecosystem functioning. Obtaining
862 such functional data at large spatial and temporal scales is necessary to raise public awareness and
863 likely capture the attention of the operational sphere, and thus promote the use of aquatic microbial
864 community functioning in regulatory frameworks (including Ecological Risk Assessment).

865

881

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