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Twenty years of research in ecosystem functions in aquatic microbial ecotoxicology

Soizic Morin and Joan Artigas

Abstract

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

Keywords: aquatic microbial ecotoxicology, community-level effects, microbial functions, pesticides.

Running head: Ecosystem functions in aquatic microbial ecotoxicology

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

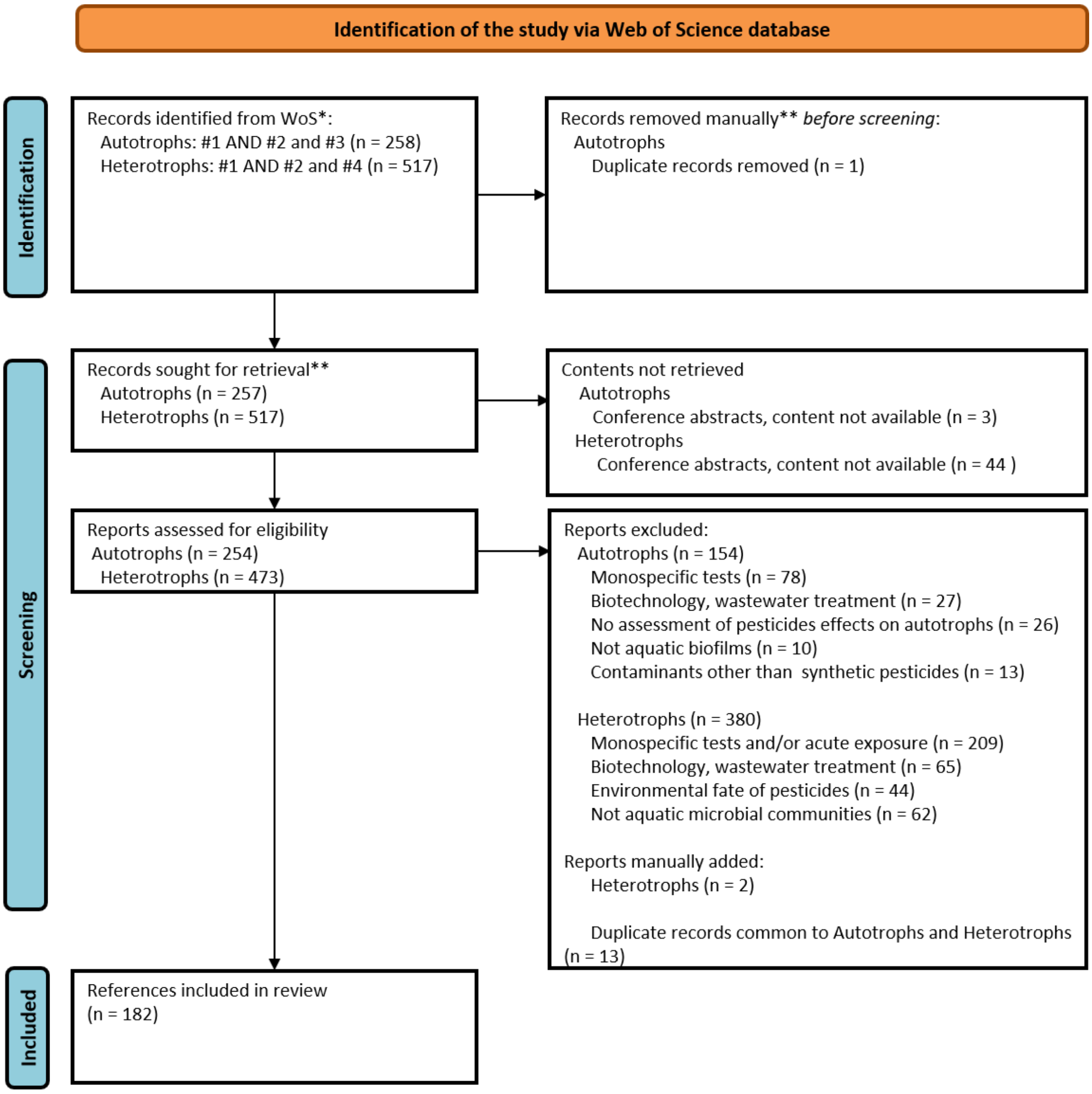

Figure 1. PRISMA 2020 flow diagram for the systematic review (Page et al. 2021). Detailed search requests are available in SI1 (*). No automation tools were used (**).

3. Results and discussion

3.1. Communities targeted and experimental conditions

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

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

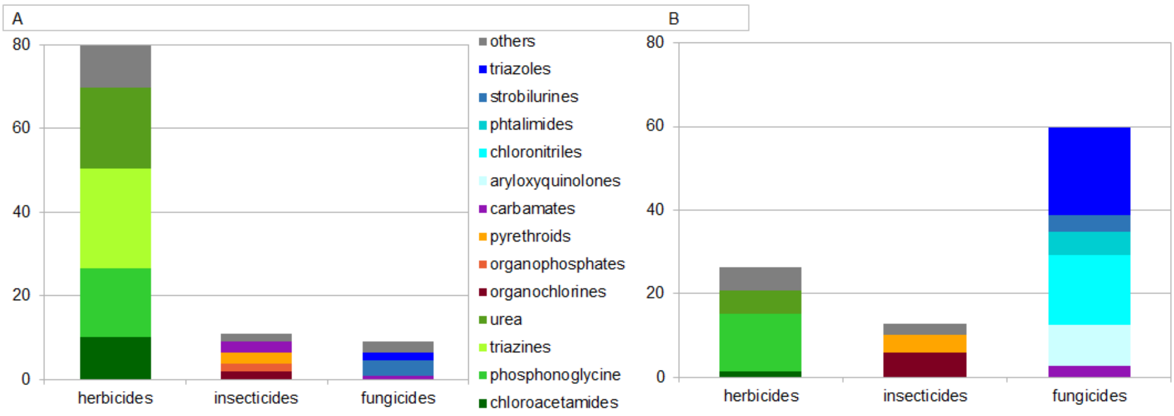

Figure 2: Chemical families of the 3 main classes of pesticides (bars = counts, 1 family may contain several molecules) tested against aquatic microbial communities under controlled conditions, excluding field and micro/mesocosm studies where complex environmental mixtures of pesticides were present. Note that several studies include 2 or more pesticide molecules, sometimes

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

3.2. General overview of functional endpoints affected by pesticide exposure

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

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

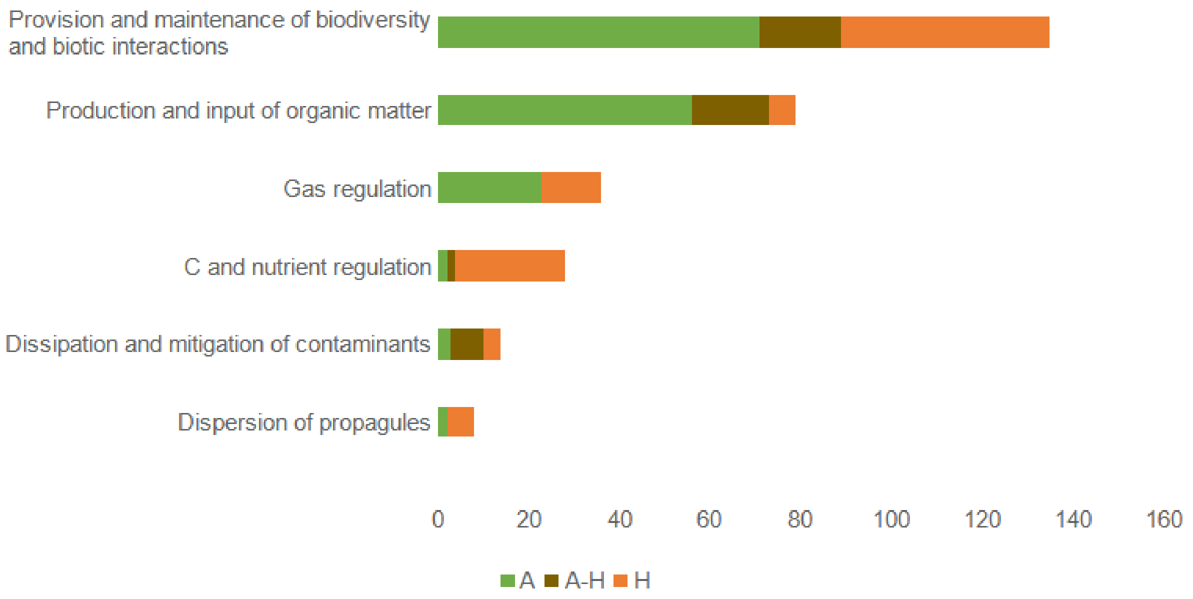

Figure 3. Number of AME studies examining several ecosystem functions between 2000 and 2020 for autotrophic (A), heterotrophic (H), or both (A-H) components of aquatic microbial communities (n=168). Note that several functions may be considered in a single record.

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).

Table 2. Main ecosystem functions, processes and endpoints measured in microbial community ecotoxicology studies during between 2000 and 2020. 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 references (non-exhaustive) using the methods are provided, together with the main observed effects of pesticide exposure towards the corresponding 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

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

Provision and maintenance of biodiversity

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

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

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

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

Provision and maintenance of biotic interactions

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

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

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

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

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

Gas regulation

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

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

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

Nutrient regulation

The impacts of pesticides on the function of regulation of nutrient cycles relative to freshwater microalgae are mentioned, for example in the review by Debenest et al. (2010). Some herbicides can reduce the absorption of nutrients (nitrates, nitrites, phosphorus and silica, in particular). In their review of the environmental consequences of herbicide impacts on cyanobacteria, Brêda-Alves et al. (2021) showed that increasing concentrations of several herbicides in aquatic environments impair atmospheric nitrogen fixation by cyanobacteria, as a result of photosynthesis inhibition. Such a decrease is likely to impact the overall aquatic nitrogen cycle. However, in their study addressing the effects of atrazine on laboratory periphyton, Murdoch et al. (2013) observed an early, sharp reduction of NH4+ uptake rates (75 to 84%) after 3 days of exposure, followed by a recovery of control rates after 1 week. According to these observations, the effects of pesticide exposure on nutrient cycles may be transient. Concerning P cycle, Proia et al. (2011) did not observe any change in phosphorus uptake by periphyton exposed for 2 weeks to diuron.

Dissipation and mitigation of contaminants

Living in biofilms can buffer microorganisms from variations in the external environment, including exposure to pesticides. Besides, autotrophic biofilms can bioaccumulate, and thus partly remove, organic pesticides from water. In their recent review, Bonnineau et al. (2021) demonstrated that freshwater periphyton can accumulate organic pesticides present in the water column with bio-uptake efficiencies varying according to their hydrophobicity, estimated by their partition coefficient between octanol and water (log Kow). Besides, Vonk & Kraak (2020) reported that uptake rates are influenced by biological characteristics (cell size and lipid composition of the organisms). Bioconcentration factors are calculated as the ratio of pesticide concentration in the biofilm with respect to its concentration in surrounding water, and values over 1 indicate bioconcentration. In Canadian wetlands (Rondeau Bay, Ontario), Rooney et al. (2020) found that periphyton bioconcentrates a large variety of pesticides, with bioconcentration factors ranging from 12 for the herbicide dicamba up to 6864 for the fungicide boscalid. Removal of pesticides by microalgae from their environment was also shown to occur in phytoplankton. In lakes, high bioconcentration factors for organochlorine pesticides were detected in phytoplankton (Zhao et al. 2015; Qiu et al. 2017), with a high affinity of pesticides for diatoms and cryptophytes. However, bioconcentration of pesticides in primary producers may expose other aquatic biota via consumption (Qiu et al. 2017; Rooney et al. 2020), and therefore have deleterious consequences on biotic interactions (“Provision and maintenance of biotic interactions”).

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

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

-Provision and maintenance of biodiversity and trophic interactions, highlighting the selection of tolerant microalgae (taxa or groups) and a decrease in diversity, while cascading effects were generally weak or hardly observable. However, the current development of dietary tracers (e. g. fatty acids) is a promising approach to shape future research;

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

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

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

Provision and maintenance of biodiversity and biotic interactions

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

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

Production and supply of organic matter

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

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

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

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

Gas regulation

The assessment of the impact of pesticides on microbial gas exchanges with the atmosphere has focused mainly on the carbon and nitrogen cycles (e. g. Widenfalk et al. 2004, Chen et al. 2019). Studies assessing the impact of pesticides on the sulfur- and methane gases are rare in literature (e. g. Garcia-Cruz et al. 2010). The study of endpoints related to respiration and denitrification processes is the most used in aquatic microbial ecotoxicology. Respiration and denitrification appeared less responsive to pesticides in aquatic environments with an equal number of studies showing negative or neutral effects. Respiration rates in sediment microbial communities from a reservoir system in China decreased as a function of a gradient of fungicide (chlorothalonil) and insecticide (propetamphos) concentrations (Su et al. 2019, Chen et al. 2019). Similar results were observed in sediment microbial communities from an estuary zone in England (Garcia-Ortega et al. 2011). The exposure history of microbial communities to pesticide contamination appears to determine the response of respiration to the fungicide tebuconazole (Artigas et al. 2014). For instance, periphyton respiration rates decreased after exposure to environmental concentrations of tebuconazole in a site less contaminated by pesticides than in a site more contaminated by pesticides. Substrate-induced respiration (SIR) is one of the most frequently used techniques to estimate respiration rates in microbial communities (e. g. Tlili et al. 2011). It is important to note that SIR measurements include the contribution of both autotrophs and heterotrophs in the respiration process, and therefore caution must be taken when distinguishing pesticide effects between components of the microbial community. The accumulation of high concentrations (mg/L) of metabolites from the herbicide 2,4-D (4-chlorophenol, 2-chlorophenol and phenol) can inhibit the respiration rates from biofilms dominated by sulfur-reducing bacteria (Garcia-Cruz et al. 2010). High concentrations of the herbicide glyphosate can display neutral or stimulating effects on the respiration rates of microbial communities from streams (Artigas et al. 2020) and ditch systems (Mbanaso et al. 2014), respectively. Neutral effects of the herbicide diuron and the insecticide imidacloprid were observed in communities from epilithon (Pesce et al. 2006) and associated with decomposing leaf litter (Kreutzweiser et al. 2007) in streams, respectively. Laboratory studies (Widenfalk et al. 2004) and field studies (Pesce et al. 2008) have coincided in describing the neutral effects of pesticide cocktails (composed of fungicides, herbicides and insecticides) on the respiration of heterotrophic microbial communities, as previously mentioned for autotrophs (see 3.3).

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

Carbon and nutrient regulation

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

Studies show that the decomposition of leaf litter by microbial heterotrophs is sensitive to pesticides, and only a few studies show neutral effects. Negative effects on litter decomposition were observed when fungicides are applied, probably due to their direct effect on aquatic hyphomycete communities responsible for leaf decomposition (Artigas et al. 2012b; Fernandez et al. 2015; Gardestrom et al. 2016; Dawoud et al. 2017; Feckler et al. 2018b; Rossi et al. 2018). This is the case for environmental concentrations of azoxystrobin and tebuconazole which were able to decrease fungal biomass, litter decomposition rates and cellulolytic enzyme activities (β-glucosidase, βxylosidase ou cellobiohydrolase) (Gardestrom et al. 2016; Dawoud et al. 2017; Artigas et al. 2012b). However, the extent of the effect of fungicides on microbial OM decomposition depends on: i) the type of aquatic ecosystem studied (Dimitrov et al. 2014; Donnadieu et al. 2016; Pesce et al. 2016), ii) the exposure history of communities to contamination (Gardestrom et al. 2016), iii) the OM quality (Artigas et al. 2012b), and iv) the availability of dissolved nutrients in stream water (Rossi et al. 2018). Tebuconazole can also increase certain ligninolytic enzyme activities during leaf decomposition, not because of the degradation of leaf polymers, but probably because of the detoxification and/or biodegradation mechanisms employed by heterotrophic components of microbial communities (Rossi et al. 2018; Artigas et al. 2017). The impact of herbicides (glyphosate, Kennedy et al., 2012) and insecticides (imidacloprid; Kreutzweiser et al. 2007, 2008) have been shown to weakly affect microbial litter decomposition, even if slight changes in fungal biomass accumulation and/or communities' composition (fungal and bacterial) are observed.

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

The impact of pesticides has been assessed on the diversity of carbon substrates utilisation by bacterial communities from rivers (Foley et al. 2008), wetlands (Sura et al. 2012; Lv et al. 2017) and coastal ecosystems (Pringault et al. 2016). Two herbicide cocktails, one composed of 2,4-D, MCPA, dicamba, clopyralid, dichlorprop, mecoprop, bromoxynil, glyphosate, and the other composed of diuron, di-isopropyl-atrazine, 3,4-dichlorophenylurea, alachlor and linuron, altered the metabolic diversity of biofilm communities from two wetlands in Manitoba, Canada (Sura et al. 2012) and strongly reduced the carbohydrate utilisation (and increased that of amino acids and polymers) of bacterioplankton communities from a coastal bay in Tunisia (Pringault et al. 2016). Exposure to environmental concentrations of the herbicide acetochlor increased the overall quantity, but not diversity, of carbon substrates utilisation by freshwater bacterial communities from US streams (Foley et al. 2008). Instead, the fungicides imazalil and tebuconazole did not affect the metabolic diversity of sediment bacterial communities from constructed wetlands in Denmark (Lv et al. 2017). Few ecotoxicology studies have shown the impact of pesticides on metabolic diversity, which could be explained by certain methodological constraints when measuring the use of carbon substrates in heterotrophic microbial communities (see Preston-Mafham et al. 2002). For instance, plating may induce a strong selection pressure in microbial species which would not reflect the actual metabolism of microbial communities in the field. Moreover, the tetrazolium dye immediately introduces some bias since not all bacteria nor fungi can reduce it, hence the plates do not necessarily give a complete picture of the microbial metabolism.

Dissipation and mitigation of contaminants and wastes

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

Dispersion of propagules

The production of aquatic fungi in AME studies is mostly analysed through the counting and taxonomic identification of conidia produced by aquatic hyphomycete communities (Gessner and Chauvet 1994). The sporulation rates of hyphomycetes are quite sensitive to pesticides, especially fungicides, with half of the studies showing a negative impact on this functional endpoint (e. g. Zubrod et al. 2011; Dimitrov et al. 2014; Pimentao et al. 2020) and the other half showing neutral and/or positive effects (e. g. Dawoud et al. 2017). A microcosm study showed that repeated applications of the fungicide azoxystrobin decreased the sporulation rates of the hyphomycete community colonizing black alder leaves, and this decrease was more marked in fungal assemblages from uncontaminated forest streams compared to those from pesticide-contaminated sites (Gardestrom et al. 2016). The effect of the fungicide tebuconazole on hyphomycetes sporulation varied between studies, with some clear negative effects on one side (Zubrod et al. 2011; Dimitrov et al. 2014; Pimentao et al. 2020) but also with some examples of stimulatory effects (Donnadieu et al. 2016; Dawoud et al., 2017). A possible explanation for these contradictory results may be the concentration of tebuconazole used and/or the different community species composition between experiments.

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

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

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

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

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

3.5. Under-researched functions in AME studies

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

Similar to gas cycles, most AME studies focus on pesticide effects on macro-nutrient cycles (i. e. C and N cycles) rather than on micro-nutrient cycles (i. e. potassium, iron, calcium, ...). Moreover, the impact of pesticides on macro-nutrient cycles is often assessed via proxies (i. e. gene expression, potential enzymatic activities) rather than using tracer addition approaches (i. e. NO_3-^{15}N, Mulholland et al. 2004) which are more integrative of microbial nutrient uptake capacities. Few studies have examined the effect of pesticides on the phosphorus uptake capacity of periphyton by measuring the temporal decay of soluble reactive phosphorus (SRP) (e. g. the herbicide diuron, Proia et al. 2011). Further, AME studies could also integrate pesticides in carbon and nutrient cycling of contaminated environments since pesticides can represent a nutrient resource for aquatic microorganisms (e. g. Carles et al. 2019).

Overall, there is a lack of studies assessing the impacts of pesticides on the functions shared by autotrophic and heterotrophic microorganisms (11% of the references explicitly consider the functions as ensured by both components, see Figure 3). For instance, gas exchange (i. e. respiration), OM production, nutrient regulation (i. e. nutrient uptake or enzymatic activities) or pesticide dissipation functions measured at the community level reflect the sum of metabolic processes carried out by both autotrophs and heterotrophs in the microbial community. Further, studies at the ecosystem scale should consider the sum of metabolisms measured in the different habitats of the ecosystem. Indeed, a range of studies shows the importance of considering the metabolism from both planktonic and littoral habitats in lakes when assessing carbon fluxes at the ecosystem scale (Vadeboncoeur et al. 2001; Vesterinen et al. 2017).

Finally, the protozoan community has been largely overlooked in AME studies despite contributing to gas and nutrient regulation, production and supply of OM, and to the provision and maintenance of biotic interactions in aquatic ecosystems. While some studies have attempted to assess pesticide effect on ciliate and flagellate populations densities (e. g. Chang et al. 2011; Neury-Ormanni et al. 2016; Lu et al. 2020), studies assessing effects on ecosystem functions ensured by protozoans are missing in the literature. Lu et al. (2020) observed that abundances of eukaryotic microbes increased in the whole zooplankton community of lake Taihu (China), especially rotifers, which is in accordance with the phenomenon that rotifers increase their hatchling proportion under exposure to glyphosate (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.

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

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

Fourth, unravelling "omics" multi-functional responses of microbial communities to pesticides will allow us to detect, through non-targeted analyses, unsuspected functional responses to pesticides. The literature review of Ebner (2021) found only 648 studies addressing "omics" and "ecotoxicology" in the last twenty years (2000-2020) which is a relatively low score. This review also concludes that transcriptomics is the most frequently applied method (43%), followed by proteomics (30%), metabolomics (13%) and finally, multi-omics (combination of two or multiple omics methods, 13%). We still identify a gap in the potential use of omics data to explain multiple functions in microbial communities. Johnson et al. (2015) pioneered in suggesting an "association mining approach" between the rate constants of observed biotransformation reactions and meta-omics data as an untargeted approach to generate hypotheses about potential causal linkages between enzymes and pesticides biotransformation. Recently, Achermann et al. (2020) used association mining to demonstrate quantitative correlations between metatranscriptomic data and micropollutant biotransformation in activated sludge. We consider that extrapolation of association mining between chemical and metatranscriptomic profiling to enzymes likely involved in catalysing reactions related to important ecosystem functions, under natural conditions, would help in bridging the gap between -omics data potential and the wide diversity of functions ensured by microbial communities.

5. Concluding remarks

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

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