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Magali Brosed, Sylvain Lamothe, Eric Chauvet

To cite this version:

Magali Brosed, Sylvain Lamothe, Eric Chauvet. Litter breakdown for ecosystem integrity assessment also applies to streams affected by pesticides. Hydrobiologia, 2016, vol. 773 (n° 1), pp. 87-102. 10.1007/s10750-016-2681-2 . hal-01360658

HAL Id: hal-01360658

https://hal.science/hal-01360658

Submitted on 6 Sep 2016

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To link to this article : 10.1007/s10750-016-2681-2
URL: http://dx.doi.org/10.1007/s10750-016-2681-2

To cite this version : Brosed, Magali and Lamothe, Sylvain and Chauvet, Eric *Litter breakdown for ecosystem integrity assessment also applies to streams affected by pesticides.* (2016) Hydrobiologia, vol. 773 (n° 1). pp. 87-102. ISSN 0018-8158

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Litter breakdown for ecosystem integrity assessment also applies to streams affected by pesticides

Brosed Magali · Lamothe Sylvain · Chauvet Eric

Abstract While the impact of various anthropogenic alterations, e.g. nutrient enrichment, has been documented on leaf litter breakdown—a key process for stream ecosystems—, our objective was to assess the response of this process to pesticides in agricultural streams. We hypothesized the impairment to be correlated with the pesticides contamination gradient, and the invertebrate decomposers to be more affected than microbial ones. Alder total breakdown rate was found to strongly decrease along the pesticide concentration in 12 French streams, only due to invertebrate-driven breakdown (as determined in coarse-mesh bags) since microbial-driven breakdown (fine-mesh bags) remained unchanged. Coherently, litter-associated shredder taxa richness and abundance together with $SPEAR_{pesticide}$ (a specific indicator based on invertebrate traits) were greatly reduced, whereas pesticide toxicity did not affect litter-associated fungal biomass and taxa richness. Consequently, the presence of pesticides compromised leaf breakdown, as microbial decomposers did not compensate for the invertebrate decomposers decline. This occurred while pesticides concentrations even in the most contaminated stream were under the European Union's Uniform Principles thresholds for targeted species. Our study showed that litter breakdown, particularly the ratio of total to microbial-driven breakdown rate, is a pertinent proxy to assess the functional integrity of pesticide-contaminated streams.

Keywords Decomposition rate · Fungi · Shredders · $SPEAR_{pesticide}$ · Toxic units

Electronic supplementary material The online version of this article (doi:10.1007/s10750-016-2681-2) contains supplementary material, which is available to authorized users.

Handling Editor: Verónica Jacinta Lopes Ferreira

B. Magali (✉) · L. Sylvain · C. Eric
EcoLab, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France
e-mail: magali.brosed@gmail.com

B. Magali
ASCONIT Consultants, 7 rue Hermès, ZAC du Canal, Bât. A, 31520 Ramonville Saint-Agne, France

Introduction

Due to modifications directly or indirectly caused by human activities, the evaluation of ecosystem functions in streams and rivers has become an important objective to reach, in complement to current bioassessment tools mainly based on fish, macrobenthos, diatom and macrophyte communities (Reyjol et al., 2014). Ecosystem functions correspond to the flow of matter and energy regulated by biotic and abiotic factors occurring in the ecosystem itself and its adjacent environments. Assessing stream functions thus respond to a holistic approach of ecosystem

health evaluation. It relies on a variety of biological processes, by focussing on the quantification of fluxes and rates and integrating both a temporal dimension and the activities of organisms at different levels of organization (Bunn & Davies, 2000; Palmer & Febria, 2012). A number of ecosystem-level processes have been proposed for stream bioassessment, encompassing ecosystem metabolism (gross primary production and community respiration), leaf litter breakdown and nutrient cycling (Gessner & Chauvet, 2002). Among these, leaf litter breakdown has generated many studies focused on a variety of functional impairments, for instance, regarding stream water quality, channel hydromorphology and riparian vegetation (e.g. Lecerf & Chauvet, 2008; Kominoski et al., 2011; Woodward et al., 2012; Elosegi & Sabater, 2013). Leaf litter breakdown is a key process for stream ecosystem sustainability as a driver of matter and energy for the aquatic food web derived from terrestrial sources. By implying both macroinvertebrates and microorganisms, this process results from the activity of a wide variety of organisms with diverse traits and biological requirements and potentially exhibiting differential sensitivity to the functional impairments. Gessner & Chauvet (2002) have proposed to discriminate invertebrate-driven and microbial-driven breakdown to improve robustness and sensitivity of litter breakdown assays.

Detection of pesticides and their transformed products in aquatic ecosystems is difficult, not only due to the diffuse character of this contamination of agricultural origin and their widespread occurrence, particularly that of herbicides, but also to the very low concentrations found in natural waters (most often $<1\ \mu g\ l^{-1}$). In addition, field studies may fail to determine specific effects of pesticides, simply because they are mixed with confounding factors (e.g. heavy metals; Cheng et al., 1997; Piscart et al., 2011). Therefore, sampling and analysing all ecotoxicologically relevant substances entering a water body are challenging. The assessment is further complicated by the scarcity of data on potential effects of individual (and mixed) compounds on species in the target system. Finally, confounding effects on aquatic communities from other anthropogenic stressors may also co-occur in agricultural areas (e.g. riparian forest clearcutting, stream channelization and nutrient enrichment). On one hand, conventional bioassessment indices for water quality fail to attribute any observed changes in biological communities to pesticide contamination (e.g. Beketov et al., 2009). On the other hand, a specific indicator based on functional traits of invertebrates, the SPEcies At Risk indicator for pesticides ($SPEAR_{pesticide}$), has proven its efficiency to detect the effects of pesticide contamination on aquatic taxa (Liess & Von der Ohe, 2005; Schäfer et al., 2007). This indicator uses both physiological and biological traits (sensitivity to toxicants; generation time, dispersal capacity and length of life stages outside the aquatic habitat, respectively) to determine the fraction of the abundance of sensitive taxa in communities. $SPEAR_{pesticide}$ has been shown to be relatively constant over reference sites from different ecoregions and to respond to pesticide stress (Liess & Von der Ohe, 2005; Liess et al., 2008; Von der Ohe & Goedkoop, 2013), while the effects detected by this metric have been shown to translate into losses of regional biodiversity (Beketov et al., 2013). In addition, macroinvertebrates may be influenced by pesticides in their physiological and behavioural activity, not only through exposure to the water phase but also via food uptake. As an illustration, insecticide exposure at field relevant concentrations has been shown to cause feeding inhibition of mayflies and oligochaetes (Alexander et al., 2007). Feeding inhibition in *Gammarus* subjected to short-term pulse exposure of a pesticide mixture may be strongly detrimental to the energy transfer along the food chain in the aquatic ecosystem (Bundschuh et al., 2013).

In contrast to individual or community scales, no metrics have been applied to assess the impact of pesticides on ecosystem-level processes. Meta-analyses revealed that leaf breakdown tends to be more sensitive to pesticide contamination than other functional endpoints, such as gross primary production and ecosystem respiration (Schäfer et al., 2012a; Peters et al., 2013). Individual studies that have examined litter breakdown reported contrasting results about the origin of loss in process rate, depending on whether it was mostly related to a decrease in the activity of macroinvertebrate (Schäfer et al., 2007, 2012b) or microbial (Rasmussen et al., 2012a) decomposers. A recent meta-analysis has shown that streams with very low nutrient concentrations were more sensitive to nutrient enrichment than streams exhibiting medium to high concentrations (Ferreira et al., 2015). While nutrients may be limiting in oligotrophic waters where even low nutrient increments may stimulate leaf

decomposers, particularly aquatic fungi, nutrient saturation occur in eutrophic rivers, often in interference with compounds with detrimental effects such as pesticides and heavy metals, resulting either in the absence of positive influence of nutrients or in a substantial abatement of breakdown rates (Woodward et al., 2012). A similar saturation pattern is not expected with pesticides as their concentrations in natural waters, even when considering additive effects of various compounds, generally lie by several orders of magnitude below the acute lethal concentrations determined for aquatic organisms such as *Daphnia magna* Straus or green algae (Brock et al., 2006; Schwarzenbach et al., 2006). A unimodal negative relationship between pesticides toxicity and leaf breakdown rate is thus predictable.

Our study was based on a correlative approach where we investigated the relationship of pesticide toxicity with leaf litter breakdown and leaf-associated decomposers in 12 agricultural streams of southwestern France, which were selected along a gradient of pesticide concentrations but otherwise exhibiting similar characteristics. Leaf breakdown was measured using leaf bags with large or fine mesh (FM), allowing to discriminate the contribution of macroinvertebrates and microbial decomposers, respectively. This assessment was performed in late winter, at the end of a year of pesticide monitoring using grab water sampling, with the objective to detect long-term pesticide effects on ecosystem functional endpoints. Concentrations in pesticides were expressed in terms of toxic unit (TU) to model organisms (Backhaus & Faust, 2012). We hypothesized that (1) total breakdown rate was depressed at the most contaminated sites, (2) invertebrate-driven breakdown was more affected than microbial-driven breakdown due to the higher sensitivity of leaf-shredding invertebrates compared to leaf-colonizing fungi in our streams where herbicides dominated and (3) the abundance and activity of macro- and micro-decomposers were consistent with the response of breakdown rate, as their density and activity were assumed to be similarly impacted by pesticide toxicity. Our underlying motivation was to determine the extent to which the information drawn from leaf breakdown and associated parameters is complementary to the dedicated index $SPEAR_{pesticide}$, when the objective is to evaluate the functional status of stream ecosystems impaired by pesticides.

Methods

Study sites

Our sites were selected in the Côteaux Aquitains (southwest of France), a relatively homogeneous European hydroecoregion in terms of geologic substratum (detritic materials), topography (flatland) and climate (Mediterranean and oceanic influences) (Wasson et al., 2002). Agriculture is the dominant landuse with small forest patches and some urban areas being embedded in the landscape. Our 12 surveyed sites corresponded to permanent, second–fourth order streams regularly monitored by the Adour-Garonne Water Agency for water quality, including pesticides concentration. Except for pesticide load, they exhibited similar characteristics, rather typical of lowland agricultural streams (Table 1). None of them was subject to dredging or weed cutting during the study period. Our selection of streams covered a low-to-high range of pesticide toxicity (see below).

Physical and chemical analyses of stream water

All stream water characteristics, including pesticide concentrations but excluding temperature, were obtained from the Adour-Garonne Water Agency database. Physical and chemical characterization was made at several occasions over 1 year (March 2012–March 2013), including in spring when pesticide concentrations were the highest. Determinations comprised pH, conductivity and concentrations of inorganic nutrients (nitrate, nitrite, ammonium and orthophosphate) and dissolved oxygen. Among the 139 pesticides monitored, 30 were detected in our streams including 16 herbicides, 12 fungicides and 2 insecticides. The sum of pesticide concentrations ranged from 0 to 31.11 $\mu g\ l^{-1}$, and the number of detected pesticides at each site and sampling occasion from 0 to 21 (Table S1). Pesticide toxicity at each site was determined as the maximum TU (mTU) calculated according to the following formula:

$$\mathrm{mTU} = \log\left(\max_{i=1}^{n}\left(\frac{c_i}{\mathrm{EC50}_i}\right)\right),$$

where c_i is the concentration of pesticide i, $\mathrm{EC50}_i$ is the corresponding 48–96 h median effect concentration for a given standard test species and n is the

Table 1 Location and physicochemical characteristics of stream water of the 12 sites ranked according to the increasing pesticide toxicity ($mTU_{D.\ magna}$)

	S1	S2	S3	S4	S5	S6
Longitude	43°34′29	43°46′19	43°17′32	43°54′52	43°47′34	43°23′02
Latitude	01°02′18	02°00′02	00°37′51	01°45′59	00°49′03	01°10′25
Stream order	3	3	2	3	3	4
Elevation (m)	149	157	283	185	159	213
Substrata	C, Sa	G, M, P	C, G, P	Si	G	G, P, Sa
Temperature (°C)	8.1 (4.3–12.4)	7.6 (4.8–10.3)	6.5 (2.3–11.3)	5.7 (2.0–9.5)	8.0 (4.6–12.0)	7.7 (3.3–12.9)
pH	8.2 (7.8–8.4)	8.1 (7.9–8.3)	7.9 (7.2–8.7)	7.8 (7.7–7.9)	8.3 (7.8–8.7)	8.0 (7.7–8.4)
Conductivity ($\mu S\ cm^{-1}$)	523 (79–782)	681 (628–719)	175 (134–227)	577 (534–621)	799 (735–861)	439 (285–685)
$[O_2]$ ($mg\ l^{-1}$)	8.6 (4.5–13.7)	11.4 (9.1–13.0)	10.4 (7.5–12.2)	9.0 (5.3–12.6)	8.2 (5.5–11.9)	9.9 (6.1–14.1)
$[N\text{-}NO_3]$ ($mg\ l^{-1}$)	1.05 (0.41–1.72)	9.70 (6.5–14.45)	0.81 (0.23–2.71)	1.47 (0.11–5.13)	8.76 (1.6–15.47)	1.72 (0.45–5.87)
$[N\text{-}NO_2]$ ($mg\ l^{-1}$)	0.02 (0.02–0.04)	0.03 (0.02–0.05)	<0.01 (<0.01–0.01)	<0.01 (<0.01–0.02)	0.02 (0.02–0.03)	0.02 (<0.01–0.03)
$[N\text{-}NH_4]$ ($mg\ l^{-1}$)	0.07 (0.04–0.12)	0.03 (0.02–0.04)	0.04 (0.04–0.04)	0.03 (<0.01–0.05)	0.03 (<0.01–0.04)	0.10 (0.04–0.32)
$[P\text{-}PO_4]$ ($mg\ l^{-1}$)	0.03 (0.03–0.04)	0.02 (<0.01–0.04)	0.02 (0.02–0.02)	0.02 (<0.01–0.03)	0.03 (<0.01–0.04)	0.03 (0.02–0.05)
$mTU_{D.\ magna}$	−5.60	−5.05	−4.84	−4.77	−4.64	−3.92
$sTU_{D.\ magna}$	−5.20	−4.83	−4.46	−4.44	−4.49	−3.72
mTU_{algae}	−3.66	−4.89	−0.43	−2.14	−3.68	−0.53
sTU_{algae}	−3.59	−4.82	−0.43	−2.14	−3.52	−0.51
$SPEAR_{pesticide}$	56.24	44.50	47.14	53.88	45.38	37.99
	S7	**S8**	**S9**	**S10**	**S11**	**S12**
Longitude	43°38′48	43°41′53	43°33′57	43°39′04	44°02′40	43°55′26
Latitude	01°29′02	00°02′40	01°52′55	−00°07′23	00°58′10	01°21′46
Stream order	4	4	4	4	3	4
Elevation (m)	137	108	192	133	105	89
Substrata	G, P, Si	C, G, Sa	B, C, P, Sa	P	G, P	C, P
Temperature (°C)	7.1 (3.3–11.2)	7.5 (3.7–12.3)	7.3 (3.7–10.1)	7.9 (3.6–13.0)	7.1 (3.0–11.6)	6.2 (2.8–9.8)
pH	7.8 (7.0–8.5)	8.1 (7.8–8.6)	7.8 (7.4–8.0)	7.9 (7.2–8.5)	7.9 (7.35–8.4)	7.9 (7.6–8.3)
Conductivity ($\mu S\ cm^{-1}$)	609 (285–826)	573 (467–641)	632 (114–803)	485 (301–588)	697 (442–853)	926 (659–1,126)
$[O_2]$ ($mg\ l^{-1}$)	7.8 (3.9–11.8)	6.7 (0.8–8.9)	8.9 (7.6–11.1)	8.8 (4.8–13.2)	8.9 (4.9–13.1)	8.8 (5.4–11.2)
$[N\text{-}NO_3]$ ($mg\ l^{-1}$)	2.53 (0.68–5.42)	2.47 (0.23–5.04)	3.63 (0.63–10.55)	5.42 (2.71–7.68)	2.61 (0.83–5.69)	2.76 (1.13–4.06)
$[N\text{-}NO_2]$ ($mg\ l^{-1}$)	0.14 (0.05–0.22)	0.07 (0.02–0.17)	0.05 (<0.01–0.12)	0.03 (0.01–0.06)	0.02 (<0.01–0.06)	0.02 (<0.01–0.04)
$[N\text{-}NH_4]$ ($mg\ l^{-1}$)	0.93 (0.20–3.27)	0.24 (0.04–1.09)	0.09 (<0.01–0.3)	0.07 (0.02–0.22)	0.04 (0.04–0.04)	0.10 (0.04–0.16)
$[P\text{-}PO_4]$ ($mg\ l^{-1}$)	0.77 (0.05–1.27)	0.05 (0.03–0.08)	0.09 (<0.01–0.16)	0.05 (0.02–0.14)	0.05 (0.03–0.1)	0.27 (0.23–0.33)
$mTU_{D.\ magna}$	−3.54	−3.33	−3.14	−2.80	−2.48	−1.81
$sTU_{D.\ magna}$	−2.95	−3.19	−3.06	−2.50	−2.30	−1.74
mTU_{algae}	−0.76	0.15	−1.75	0.65	0.80	−0.66

Table 1 continued

	S7	S8	S9	S10	S11	S12
sTU_{algae}	−0.33	0.18	−1.32	0.65	0.81	−0.48
$SPEAR_{pesticide}$	30.35	21.56	22.34	54.02	16.82	7.52

Dominant substrata consist of *C* clay, *Sa* sand, *G* gravel, *M* mud, *P* pebble, *Si* silt and *B* boulder. Water temperatures correspond to mean and range (in parentheses) values over the 21 days of leaf litter exposure. For pH, conductivity and concentrations in dissolved oxygen and inorganic nutrients, values refer to mean and range (in parentheses) values from March 2012 to March 2013. Maximum toxic unit and sum of toxicity unit maxima for *Daphnia magna* and algae together with $SPEAR_{pesticide}$ at each site are indicated

S1 Boulouze, *S2* Agros, *S3* Gimone, *S4* Tescou, *S5* Orbe, *S6* Touch, *S7* Sausse, *S8* Petit Midour, *S9* Girou, *S10* Bergon, *S11* Ayroux, *S12* Rieu-Tort

number of pesticides detected in the site (Backhaus & Faust, 2012; cf. Table S1). Due to the logarithmic transformation, TUs over different sites were within a relatively narrow range, e.g. from −5 or lower from the lowest contaminated site up to −1 or higher from the most contaminated site, with a TU of 1 meaning that the observed pesticide concentration is 10 times higher than the median effect concentration for that pesticide and the standard species considered. Alternatively, pesticide toxicity at each site was expressed as the sum of TUs (sTUs; Liess & Von der Ohe, 2005):

$$sTU = \log\left(\sum_{i=1}^{n} \frac{c_i}{EC50_i}\right).$$

Standard test species were selected among the closest organisms to those involved in the ecosystem function investigated in this study, i.e. *D. magna* ($TU_{D.\ magna}$) for invertebrate decomposers and diverse algae for microbial ones (TU_{algae}; University of Hertfordshire, 2013).

Leaf litter breakdown

In autumn 2012, alder (*Alnus glutinosa* (L.) Gaertn.) leaves were collected just after abscission from a riparian zone within the same European hydroecoregion. Leaves were air-dried for 7 days, weighed in batches of approximately 4 g (3.96–4.04 g) and enclosed in coarse mesh (CM, 9 mm opening) and FM (0.5 mm opening) bags (15 × 20 cm). Initial dry mass of leaf sample was determined by using a correction coefficient obtained from eight additional batches of 4 g dried at 105°C for 48 h.

In each stream, six experimental units consisting of one CM bag and one FM bag were anchored in the streambed in mid-February 2013. This corresponded to a period where agricultural lands (mostly devoted to the production of cereals and oleaginous plants) were often free of cultures, with pesticides, especially herbicides, being mainly applied from early spring until late summer. Boulders were added to maintain the litterbags at the stream bottom. The six experimental units were placed at a distance of >5 m from each other by selecting comparable conditions of exposure (e.g. depth and substratum) within stream riffles (=blocks) whenever possible. Riffles were preferred to pools in order to optimize fungal colonization and avoid the risk of anoxia in leaf packs resulting from fine sediment deposition. Temperature was continuously monitored in each stream using temperature loggers (HOBO UA-001-64, Bourne, MA, USA). After 21 days of exposure, leaf bags were removed cautiously, e.g. in limiting the loss of leaf-associated invertebrates, and stored in a cool box during transport to the laboratory.

Leaf litter from FM bags was gently washed under tap water to remove sediments, and ten leaf discs per sample were cut from different leaves avoiding the central vein. Five out of the ten discs were used immediately to induce sporulation of aquatic hyphomycetes and the other five discs were stored at −20°C for later fungal biomass estimation. The remaining leaves were dried at 105°C for 48 h and weighed (±0.01 g).

Leaf litter from CM bags was gently washed and leaf-associated macroinvertebrates were collected on a 500 µm sieve and stored in ethanol (70% vol/vol). Leaves were dried to constant mass at 105°C for 48 h and weighed (±0.01 g). The ash-free dry mass (AFDM) was extrapolated from aliquots of ground dry litter (0.25 ± 0.02 g) burnt at 550°C for 3 h and weighted to determine the ash content.

Leaf-associated microorganisms and macroinvertebrates

Ergosterol concentration was used as a proxy of leaf-associated fungal biomass (Gessner & Chauvet, 1993). Lipids were extracted from sets of five leaf discs by heating (80°C for 30 min) in 0.8% (mass/vol) KOH–methanol, purified by solid-phase extraction [Oasis HLB 3 cm^3 (60 mg) extraction cartridge, Waters, Milford, MA, USA], and quantified at 282 nm and 33°C using high-performance liquid chromatography on a C_{18} column with methanol as the mobile phase (Gessner, 2005). Ergosterol was converted to fungal biomass using a conversion factor of 5.5 mg ergosterol g^{-1} AFDM (Gessner & Chauvet, 1993), and biomass expressed as mg g^{-1} leaf AFDM.

For each sample, sporulation was induced from the set of five leaf discs introduced into 100-ml Erlenmeyer flasks with 20 ml of filtered stream water (GF/C, 1.2 µm pore size, Whatman, Maidstone, UK) placed on an orbital shaker at 12°C. After 48 h of incubation, the conidial suspensions together with rinsing water were adjusted to 40 ml in 50 ml polypropylene tubes and fixed with 5 ml of 37.5% formaldehyde. Aliquots of conidial suspensions were filtered (Millipore SMWP, 5 µm pore size, Billerica, MA, USA) and stained with 0.05% (mass/vol) Trypan blue in 60% lactic acid, with at least 200 conidia per replicate being identified and counted under the microscope at 100–400× (Gessner et al., 2003) using identification keys of Chauvet (1990) and Gulis et al. (2005). Leaf discs were dried at 105°C and weighted, and the sporulation rate was expressed as the number of conidia produced per μg^{-1} leaf AFDM day^{-1}.

Invertebrates were identified to the family level and assigned to functional feeding groups according to Tachet et al. (2010). Taxa were considered as shredders when their diet was at least composed of 20% of coarse particulate organic matter. The relative abundance of shredder taxa and shredder taxa richness was calculated. Abundance was expressed as number of individuals g^{-1} leaf AFDM and taxa richness and number of taxa per sample. In addition, the abundance of SPEcies At Risk of being impacted by chronically pesticide contamination ($SPEAR_{pesticide}$) was computed for each site using the freely available online SPEAR calculator (http://www.systemecology.eu/spear).

Data analysis

Exponential leaf breakdown rate (k) for each bag was derived from the decay model (Wieder & Lang, 1982) according to the formula: $k = -\ln (M_t/M_0)/t$, where M_0 is the initial AFDM, M_t is the AFDM remaining, and t is the exposure time (in days). Total (k_{total}) and microbial-driven ($k_{microbe}$) breakdown rates were calculated from CM and FM bags, respectively. Invertebrate-driven breakdown rate ($k_{invertebrate}$) was calculated from the difference between leaf AFDM remaining in CM and FM bags at each experimental unit. The $k_{total}/k_{microbe}$ ratio was used to evaluate shifts in the relative contribution of shredders and microorganisms to leaf breakdown (Gessner & Chauvet, 2002).

A Pearson correlation matrix taking into account the leaf litter breakdown rates, abundance and richness of total invertebrates and shredders, fungal biomass, conidial production and taxa richness, $SPEAR_{pesticide}$, and the expressions of pesticides contamination (mTU and sTU for both *D. magna* and algae) together with ammonium as a potentially deleterious compound (cf. Lecerf et al., 2006) was tested. Abundance of both total invertebrates and shredders was logarithmically transformed. Correlations were done using R (3.1.3 version). The significance threshold was set at $P < 0.05$.

Results

Pesticides

Our sites exhibited a wide gradient of pesticide concentrations as expressed in TUs (Table 1). Boulouze (S1) and Agros (S2) were the less contaminated sites, and Rieu-Tort (S12) and Ayroux (S11) the most contaminated sites, depending on considering toxicity to *D. magna* or algae, respectively. Over all sites, the range of pesticide contamination was wider considering algae (mTU_{algae}: −4.89 to 0.80) than *D. magna* ($mTU_{D.\ magna}$: −5.60 to −1.81; Table S1). At each site, mTU_{algae} was higher than $mTU_{D.\ magna}$, with the Gimone (S3) site exhibiting the largest discrepancy (−0.43 and −4.84, respectively). Values of the sTUs and their patterns among sites were very similar to those of mTU for both algae and *Daphnia*.

The herbicides acetochlor (chloroacetamide) and, to a lesser extent, oxadiazon (oxadiazole), isoproturon (urea) and diuron (phenylurea) were clearly the most important compounds, i.e. resulting in the highest values of mTU_{algae} (Table S1). The highest $mTU_{D.\ magna}$ values were due to the carbamate insecticide carbaryl, followed by the herbicide isoproturon.

Leaf litter breakdown rates

Five out of the 144 leaf bags deployed at our 12 stream sites [Boulouze (S1): one FM bag, Touch (S7) and Rieu-Tort (S12): one FM and one CM bag] could not be retrieved after 21 days. In CM bags, 43.3–66.1% of the initial leaf litter mass was retrieved after 21 days. This translated into total breakdown rates (k_{total}) ranging from 0.0198 ± 0.0014 day^{-1} (mean ± SE; Rieu-Tort, S12) to 0.0413 ± 0.0058 day^{-1} (Boulouze, S1, Fig. 1A). In FM bags, remaining leaf litter ranged from 59.9 to 71.0% corresponding to microbial-driven breakdown rates ($k_{microbe}$) of 0.0247 ± 0.0023 day^{-1} (Girou, S9) and 0.0163 ± 0.00005 day^{-1} (Agros, S2), respectively. As a result, invertebrate-driven breakdown rates ($k_{invertebrate}$) ranged from 0.0009 ± 0.0004 day^{-1} (Rieu-Tort, S12) to 0.0144 ± 0.0023 day^{-1} (Agros, S2), corresponding to 98–74% of initial litter mass remaining after 21 days, which underlined the almost null breakdown driven by invertebrates at the former site. Both k_{total} and $k_{invertebrate}$ exhibited a decrease along the gradient of pesticide toxicity ($mTU_{D.\ magna}$) with linear relationships being significant ($r^2 \geq 0.25$, $P < 0.0001$), whereas $k_{microbe}$ showed no clear trend (Fig. 1A). The increase in pesticide toxicity thus coincided with a sharp decrease of the total to microbial-driven breakdown rate ratio, with the linear relationship being highly significant ($r^2 = 0.30$, $P < 0.0001$; Fig. 1B). Similarly, significant patterns were also found for breakdown rates expressed in degree-day (data not shown).

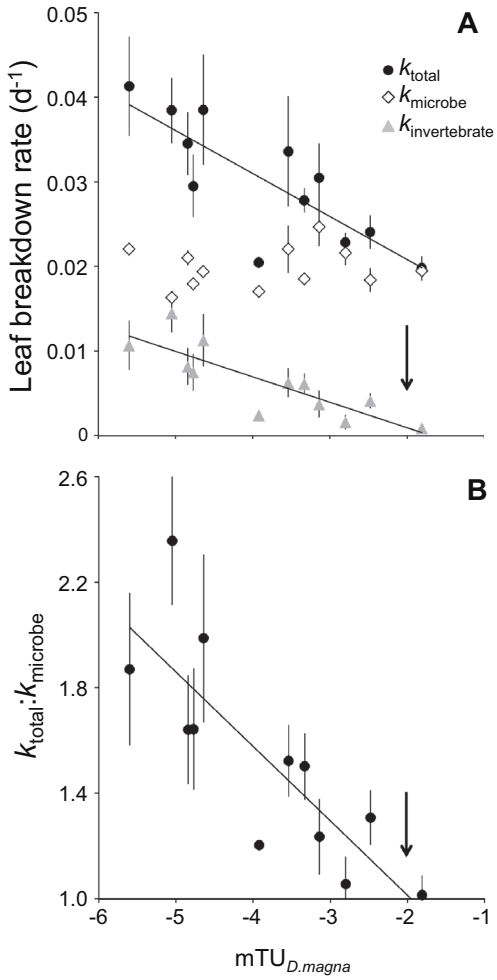

Fig. 1 **A** Total, microbial-driven and invertebrate-driven leaf breakdown rates (mean ± SE, $n = 6$) along the gradient of maximum toxic units for *Daphnia magna* encountered in the 12 sites. **B** Total–microbial-driven breakdown rate ratio (mean ± SE, $n = 6$) along the gradient of maximum toxic units for *Daphnia magna*. The linear regressions were based on all six experimental units per stream. Parameters of the linear regression for total breakdown rate are $y = -0.0051x + 0.0051$, $r^2 = 0.25$, $P < 0.0001$. Invertebrate-driven breakdown rate: $y = -0.0031x - 0.0032$, $r^2 = 0.31$, $P < 0.0001$. Total–microbial-driven breakdown rate ratio: $y = -0.2709x + 0.5204$, $r^2 = 0.30$, $P < 0.0001$. The linear regression for microbial-driven breakdown rate was not significant. The *arrow* indicates the threshold defined by the European Union as the EC_{50} of *Daphnia* divided by an assessment factor of 100 (EEC, 1991)

Leaf-associated decomposers

The fungal biomass associated with the remaining leaf litter at day 21 ranged from 54.5 ± 7.1 (Tescou, S4) to 164.0 ± 9.6 mg g^{-1} leaf AFDM (Gimone, S3) (mean ± SE), and conidial production of aquatic hyphomycetes from 0.32 ± 0.04 (Agros, S2) to 2.99 ± 2.08 conidia μg^{-1} leaf AFDM day^{-1}(Sausse, S7; mean ± SE) (Table S2). While the fungal taxa richness was around 12 species per stream (range 9–16), four of them occurred in all sites, i.e. *Alatospora acuminata* Ingold, *Flagellospora curvula* Ingold, *Tetracladium marchalianum* De Wild. and *Lemonniera aquatica* De Wild. (Table S2). Three of these species dominated the conidial production, with an average contribution of 35.8% (±6.4, SE) for *A.*

acuminata, 27.4% (±6.5) for *F. curvula* and 18.6% (±3.9) for *T. marchalianum*.

The mean (±SE) abundance of invertebrates from CM bags was 59.3 ± 11.1 individuals g^{-1} leaf AFDM, with the minimum occurring at both Ayroux (S11; 18.4 ± 3.4) and Girou (S9; 18.4 ± 4.4) and the maximum being 135.9 ± 77.7 at Boulouze (S1), i.e. the second and fourth most contaminated sites and the less contaminated site regarding toxicity to *Daphnia*, respectively (Table S3). The mean invertebrate taxa richness per site was 15 with values ranging between 8 (Rieu-Tort, S12, the most contaminated site) and 26 (Gimone, S3, the third less contaminated site). Taxa belonged to five classes (Arachnida, Clitellata, Insecta, Gastropoda and Malacostraca), with insect larvae being largely dominant. Asellidae was the only family for Malacostraca as Hydrachnidiae was for Arachnida. Gastropoda was represented by Hydrobiidae and Physidae, and Clitellata by Glossiphoniidae, Oligochaeta and Planorbidae. Chironomidae was the most abundant family, contributing in average (±SE) to 69.7 ± 6.8% of the total invertebrates [from 20.7 ± 6.4% at Gimone (S3) to 96.2 ± 13.2% at Rieu-Tort (S12)]. Baetidae was the only other taxa present in all sites, even though it only contributed to 3.2 ± 1.3% of the total on average.

The mean (±SE) abundance of shredders was 9.3 ± 6.1 individuals g^{-1} leaf AFDM, ranging from 0.4 ± 0.2 to 75.2 ± 49.7 at Rieu-Tort (S12) and Boulouze (S1), i.e. the most and less contaminated sites, respectively. In average (±SE), shredders accounted for 10.7 ± 4.5% of the total invertebrates [3.2 ± 2.4% at Rieu-Tort (S12) to 46.2 ± 5.1% at Boulouze (S1)]. Shredders were only dominant at Boulouze (S1), with Nemouridae individuals accounting for 51.1 ± 34.3% of the total invertebrates (see Table S3 for more information). Consistently, the mean individual abundance from invertebrate families at risk for pesticide ($SPEAR_{pesticide}$) ranged from 56.2 (Boulouze, S1) to 7.5 (Rieu-Tort, S12).

Among-parameters correlations

Both k_{total} and $k_{invertebrate}$ were significantly and negatively correlated with $mTU_{D.\ magna}$ (Fig. 1A) and mTU_{algae} ($r \leq -0.77$, $P \leq 0.003$; Table 2). The ratios $k_{total}/k_{microbe}$ (Fig. 1B) and $k_{invertebrate}/k_{microbe}$ (data not shown) significantly decreased along the $TU_{D.\ magna}$ contamination. Both k_{total} and $k_{invertebrate}$ were positively correlated with shredder abundance ($r \geq 0.71$, $P \leq 0.010$). Shredder taxa richness was significantly related to $k_{invertebrate}$ ($r = 0.72$, $P = 0.009$), and the relationship was close to be significant with k_{total} ($r = 0.54$, $P = 0.068$). Invertebrate and shredder abundances were also negatively affected by TUs for *D. magna* ($mTU_{D.\ magna}$ and $sTU_{D.\ magna}$; $r \leq -0.75$, $P \leq 0.005$) and shredder taxa richness and abundance were negatively correlated to TUs for algae (mTU_{algae} and sTU_{algae}; $r \leq -0.60$, $P \leq 0.040$). No significant correlation was observed between mTUs and $k_{microbe}$, neither with other variables related with aquatic hyphomycetes, i.e. fungal biomass and conidial production rate (Table S3). However, positive correlations between TUs for algae and conidial production rate occurred (mTU_{algae} and sTU_{algae}; $r \geq 0.53$, $P \leq 0.077$, Table 2), the non-significance being explained by the high conidial production rate at Orbe, a poorly contaminated site.

$SPEAR_{pesticide}$ was strongly and negatively correlated to TUs related to *D. magna* ($mTU_{D.\ magna}$ and $sTU_{D.\ magna}$; $r \leq -0.76$, $P \leq 0.004$, Table 2), and positively correlated to invertebrate and shredder taxa richness as well as invertebrate and shredder abundances ($r \geq 0.66$, $P \leq 0.018$, Table 2). $SPEAR_{pesticide}$ was not correlated with breakdown rates, but discarding a site (Bergon: S10) led to significant correlations with total and invertebrate-driven breakdown rates ($r = 0.70$, $P = 0.017$; $r = 0.73$, $P = 0.012$, respectively). Finally, no correlation was found between ammonia concentration and any response variable (Table 2).

Discussion

The rate of alder leaf breakdown in our streams was strongly affected by pesticide contamination, supporting our main hypothesis. Not only the lowest and highest concentrations expressed as TUs (for *D. magna*) coincided with the highest and lowest breakdown rates, respectively, but this pattern was also consistent along the wide gradient of pesticide concentrations found in our 12 streams. Overall, these findings were consistent with previous results by Schäfer et al. (2007), even though their 2.4-fold reduction in breakdown rate reported from farmland streams in Western France was higher than the 2.1-

Table 2 Correlation matrix between response variables (means of the six experimental units for each stream), toxicity parameters and ammonium concentration, with Pearson correlation coefficient (bottom left) and *P*-values (top right) being displayed

	k_{total}	$k_{microbial}$	$k_{invertebrate}$	Mycelial biomass	Conidial production rate	Fungal taxa richness	Invertebrate taxa richness	Shredder taxa richness
k_{total}		0.557	**<0.001**	0.214	0.559	0.455	0.412	0.068
$k_{microbial}$	0.19		0.474	0.350	0.213	0.590	0.410	0.303
$k_{invertebrate}$	**0.91**	−0.23		0.500	0.247	0.702	0.223	**0.009**
Mycelial biomass	0.39	0.30	0.22		0.622	0.385	0.129	0.742
Conidial production rate	−0.19	0.39	−0.36	0.16		0.980	0.803	0.264
Fungal taxa richness	−0.24	−0.17	−0.12	−0.28	−0.01		0.829	0.690
Invertebrate taxa richness	0.26	−0.26	0.38	0.46	0.08	−0.07		**0.009**
Shredder taxa richness	0.54	−0.32	**0.72**	0.11	−0.35	−0.13	**0.72**	
Invertebrate abundance[a]	**0.64**	0.01	**0.59**	0.52	0.06	−0.51	**0.64**	0.55
Shredders abundance[a]	**0.76**	0.11	**0.71**	0.37	−0.45	−0.38	0.28	**0.63**
$SPEAR_{pesticide}$	0.52	−0.01	0.51	0.32	−0.07	−0.24	**0.67**	**0.66**
$sTU_{D.\ magna}$	**−0.80**	0.17	**−0.84**	−0.39	0.42	0.21	**−0.6**	**−0.72**
sTU_{algae}	**−0.76**	0.18	**−0.84**	−0.01	0.56	0.25	−0.26	**−0.64**
$mTU_{D.\ magna}$	**−0.81**	0.13	**−0.84**	−0.42	0.36	0.26	**−0.59**	**−0.72**
mTU_{algae}	**−0.77**	0.13	**−0.82**	0.00	0.53	0.26	−0.22	**−0.60**
$[N\text{-}NH_4]$	0.06	0.26	−0.09	−0.04	0.53	−0.03	−0.32	−0.33

	Invertebrate abundance[a]	Shredders abundance[a]	$SPEAR_{pesticide}$	$sTU_{D.magna}$	sTU_{algae}	$mTU_{D.magna}$	mTU_{algae}	$[N\text{-}NH_4]$
k_{total}	**0.026**	**0.004**	0.086	**0.002**	**0.004**	**0.001**	**0.003**	0.847
$k_{microbial}$	0.972	0.724	0.966	0.604	0.574	0.691	0.696	0.416
$k_{invertebrate}$	**0.043**	**0.010**	0.088	**0.001**	**0.001**	**0.001**	**0.001**	0.783
Mycelial biomass	0.081	0.241	0.310	0.210	0.982	0.173	0.994	0.900
Conidial production rate	0.863	0.142	0.832	0.177	0.060	0.256	0.077	0.078
Fungal taxa richness	0.089	0.227	0.462	0.520	0.429	0.415	0.406	0.916
Invertebrate taxa richness	**0.026**	0.370	**0.017**	**0.041**	0.411	**0.042**	0.496	0.309
Shredder taxa richness	0.063	**0.027**	**0.018**	**0.009**	**0.026**	**0.009**	**0.040**	0.301
Invertebrate abundance[a]		**0.013**	**<0.001**	**0.003**	0.081	**0.001**	0.100	0.804
Shredders abundance[a]	**0.69**		**0.007**	**0.005**	**0.009**	**0.003**	**0.011**	0.523
$SPEAR_{pesticide}$	**0.88**	**0.73**		**0.004**	0.119	**0.002**	0.155	0.490
$sTU_{D.\ magna}$	**−0.78**	**−0.75**	**−0.76**		**0.003**	**<0.001**	**0.005**	0.445
sTU_{algae}	−0.52	**−0.71**	−0.47	**0.78**		**0.005**	**<0.001**	0.390
$mTU_{D.\ magna}$	**−0.82**	**−0.78**	**−0.79**	**0.99**	**0.75**		**0.008**	0.629

Table 2 continued

	Invertebrate abundance[a]	Shredders abundance[a]	$SPEAR_{pesticide}$	$sTU_{D.magna}$	sTU_{algae}	$mTU_{D.magna}$	mTU_{algae}	$[N\text{-}NH_4]$
mTU_{algae}	−0.50	**−0.70**	−0.44	**0.76**	**1.00**	**0.72**		0.491
$[N\text{-}NH_4]$	−0.08	−0.21	−0.22	0.24	0.27	0.16	0.22	

Bold cases indicate significant correlations

[a] Denotes logarithmic transformation

fold decrease observed in our streams. The higher reduction in Schäfer et al.'s study could however be explained by their slightly wider range in TUs ($mTU_{D.\ magna}$: −5.0 to −0.42), and specifically by their higher extension towards higher toxicity (−0.42) compared to those occurring in our SW French streams ($mTU_{D.\ magna}$: −5.6 to −1.8). Interestingly, our total breakdown rates for alder leaf litter (0.0198–0.0413 day^{-1}) also fit with the range reported by Schäfer et al. (2007) for the same leaf species in French streams (0.008–0.067 day^{-1}) with our range again being narrower, illustrating the relative constancy of this endpoint when measured over comparable conditions, i.e. agricultural and lowland streams.

If a $mTU_{D.\ magna}$ threshold of −3.5 is used to discriminate streams affected by pesticide input from unaffected ones (Schäfer et al., 2007), then half of our streams were impacted, resulting in a total breakdown rate average reduction of 22% in those streams. Nevertheless such a reduction, although close to the first threshold of 25% proposed in Gessner & Chauvet (2002), does not provide the highest evidence of pesticide impact on leaf breakdown. Applying the same $mTU_{D.\ magna}$ threshold to invertebrate-driven breakdown rate led to a 58% reduction of the function rate, a high value identical to that reported in Schäfer et al. (2007), indicating a severe functional impairment of the ecosystem (Gessner & Chauvet, 2002). The loss of invertebrate diversity due to pesticide contamination was reported at regional scale in Germany and France both at species and family level (e.g. with a loss of up to 42% of the recorded taxonomic pool) by Beketov et al. (2013). Here, by relying on leaf-associated invertebrates, we also show a strong depletion of both shredder taxa richness and abundance, which in our most contaminated stream represented 60 and 0.5% of the values from the less contaminated stream, respectively. The decrease in shredders contribution to the invertebrate community, directly translating into reduced leaf breakdown rate, was remarkably consistent along our contamination gradient (Table S3).

A major feature of our streams impaired by pesticides was the shift from leaf breakdown co-driven by microbial and invertebrate decomposers in non-impacted streams to the control by microbial decomposers only in impacted ones. Similarly, Piscart et al. (2011) reported unaltered rates of microbial-driven breakdown of alder leaf litter in benthic habitats of two streams affected by farming and vineyard and contaminated by pesticides and metals, in contrast with invertebrate-driven breakdown, which was severely impacted. Apart from the case where one decomposer type is specifically targeted (e.g. invertebrates by insecticides), very few examples, where one type of decomposer is affected by a stressor, while the other remains unchanged, are reported in the literature. In the most documented case, e.g. nutrient enrichment of stream water, the response of decomposers in leaf litter breakdown is not unimodal. Fungi tend to be stimulated and invertebrates unchanged under low–moderate nutrient enrichment (Ferreira et al., 2006), while high eutrophication leads to unaffected or decreased fungal activity and strongly depressed invertebrate contribution (Baldy et al., 2007; Woodward et al., 2012). Compensatory contributions of both decomposer types resulting in unaltered total breakdown rates have been reported for the clearance of woody riparian vegetation in the European streams (Hladyz et al., 2010). In the present study, the response of both microbial and invertebrate decomposers over the whole pesticide gradient was remarkably unimodal. At least for invertebrates, this is supported by the constant decrease in both abundance and numbers of taxa with increasing pesticide toxicity, resulting in their gradually lower involvement in leaf breakdown. These effects were observed across various situations where pesticides were present in

mixtures of compounds differing in identity, type, concentration and toxicity. Even though it is difficult to draw any conclusion from such naturally complex conditions, these patterns suggest a potentially high sensitivity of invertebrates to the three pesticide types (herbicide, insecticide and fungicide) prevailing in our streams. When considering effects on litter-associated insect taxa, like the midges Chironomidae, our records suggest that insecticides mixtures were even more toxic than determined on the standard species *D. magna*. As an example, the EC_{50} of imidacloprid for *Chironomus tentans* Fabricius (3.4 μg l^{-1}, ECOTOX database) was 27,070 times lower than for *D. magna*, potentially resulting in higher TUs than indicated in Table 1 for four of our streams [Sausse (S7), Bergon (S10), Ayroux (S11), and Rieu-Tort (S12)]. Similarly, relying on the lowest reported EC_{50} of carbaryl, i.e. the pesticide that determined the highest TU for *D. magna* in our study [−1.81, Rieu-Tort (S12)], for *C. tentans* (1.6 μg l^{-1}, ECOTOX database), would lead to an even higher mTU (−1.20) for invertebrates in this stream.

The higher sensitivity of invertebrates can be due to the conjunction of direct (i.e. exposure to toxicant) and indirect (i.e. feeding on contaminated mycelia and leaf matter) effects like documented in Flores et al. (2014). Actually, our experimental design did not allow to discriminate the consequences of shredder taxa loss and altered feeding activity, both of which potentially resulting in depressed leaf consumption. If the drastic decrease in shredder abundance at our contaminated sites could, per se, explain most of the reduction of litter fragmentation, an inhibition of the feeding activity of shredders that survived pesticide effects cannot be precluded. Bundschuh et al. (2013) reported that the amphipod *Gammarus fossarum* Koch when briefly exposed to pesticide mixture at field relevant concentrations, i.e. with the same order of magnitude as those occurring in our streams, exhibited a 35% decreased feeding rate on alder leaf litter (low exposure scenario). A stonefly, *Pteronarcys comstocki* Smith, showed a feeding rate on alder leaves impaired by 27% in outdoor streams, due to low-concentration pulses of the insecticide imidacloprid (Pestana et al., 2009). Similarly, although not related to pesticide but cadmium exposition, increasing concentrations of this stressor resulted in decreased alder leaf mass loss and decreased feeding by a caddisfly shredder, *Sericostoma vittatum* Rambur, and a midge collector, *Chironomus riparius* Meigen (Campos et al., 2014). Importantly, *C. riparius* was reported to consume alder leaves in the absence of shredders, and thus to switch from collector to shredder feeding mode. Such sub-lethal effects with direct impact on leaf processing rate may have occurred in our study, where chironomids dominated invertebrate assemblages, especially in the most contaminated streams. The extent of which the depressed feeding activity of shredders in our contaminated sites contributed to the observed reduced breakdown rate however remains difficult to quantify, given the mixture of potentially inhibiting compounds complicated by the interactions among leaf consumers. Such an inhibitory effect, synergistically combined with the altered feeding activity caused by a contaminated leaf resource (Bundschuh et al., 2013; Flores et al., 2014), is however susceptible to occur in the frequently encountered situations where pesticide toxicity do not compromise invertebrate survival.

In contrast, fungal decomposers appear to be tolerant to a wide range of molecule types and concentrations. In particular, leaf-associated fungi are poorly sensitive to insecticides (e.g. Dalton et al., 1970), at concentrations where invertebrate detritivores are strongly if not totally repressed (Suberkropp & Wallace, 1992; Kreutzweiser et al., 2009; Thompson et al., 2015). Based on the toxicity to the used standard test organisms, herbicides were the predominant pesticides in our streams. Mecoprop was found to negatively affect leaf-associated biomass of an aquatic hyphomycete species (Bermingham et al., 1998), but this effect occurred at concentrations at least >1000 times higher than those occurring in our streams (Table S1). Two herbicides, paraquat and 2,4-DB, did not inhibit the growth of three aquatic hyphomycetes at concentrations below 10 mg l^{-1} (Chandrashekar & Kaveriappa, 1989). Freshwater hyphomycetes appear to be relatively tolerant to glyphosate (the second most frequent pesticide in our streams). Fungicides generally produced effects on fungal activity and leaf breakdown at concentrations lower than the other pesticides, but these concentrations were again found to be higher than those in our agricultural streams (Chandrashekar & Kaveriappa, 1989). In contrast with our study, Rasmussen et al. (2012b) reported a 50% reduction in microbial leaf breakdown rate in streams affected by a mixture of pesticides comprising fungicides at relatively high toxicity levels. However, this result relied

on reference streams located in forested areas versus impacted streams in agricultural areas, thus differing from our study all based on the agricultural streams. We cannot thus preclude that background levels of toxicants (as well as other stressors) occurred even in our less impacted streams, which are normally not present in forested streams. An additional factor of discrepancy in Rasmussen et al. (2012a) is the nature of leaf litter, beech, a refractory species compared to the labile alder. Refractory leaf species are often reported to exacerbate the response to stressors (e.g. Lecerf et al., 2005). Leaf-associated microorganisms in Rasmussen et al. (2012a) were limited in assimilable carbon resource, which could have made them more sensitive to pesticide contamination leading to lower k_{microbe} in contaminated streams. Moreover, due to their slower breakdown, the exposure of beech leaf litter in the contaminated stream was longer, further enhancing the response to contamination. Artigas et al. (2012) reported impaired structure and functioning of leaf-associated microbial communities due to tebuconazole, a fungicide also found in our streams but at concentrations ca. 50 lower (Table S1). Our streams contained four compounds (azoxystrobin, carbendazim, epoxiconazole and tebuconazole), which, based on the same extrapolation as in Rasmussen et al. (2012a) for fungicide toxicity to aquatic fungi (Dijksterhuis et al., 2011), exhibited moderate toxicity to fungi within a relatively narrow range across our sites ($\mathrm{mTU}_{\mathrm{fungi}}$: −2.53 to −2.09). These intermediate values are consistent with the apparently unaltered fungal activity in our study, being further supported by a plausibly higher resistance of both structure and functioning of aquatic hyphomycete to fungicides compared to non-microbial communities (Maltby et al., 2009). In addition, the absence of effects on fungal decomposers may simply result from our study period, i.e. late winter, as fungicides are predominantly applied during spring and summer (e.g. Fernández et al., 2015) most likely resulting in the strongest effects during that time, combined with a possible higher resilience of aquatic fungi due to their short life-cycle. Nevertheless, in the absence of extensive data on aquatic hyphomycete sensitivity to fungicides, it must be pointed out that standard algae toxicity tests and even the existing fungal data in the literature (Dijksterhuis et al., 2011; aquatic fungi, but not aquatic hyphomycetes) may not reflect fungicide toxicity to leaf-associated fungal decomposers and should be used with caution. As an illustration, a recent study showed microbial endpoints related to leaf litter breakdown to be drastically more sensitive towards fungicides than standard algae toxicity tests (Zubrod et al., 2015). Relying on total fungicide concentrations instead of $\mathrm{mTU}_{\mathrm{algae}}$ did not however result in any significant correlation against microbial/fungal endpoints of our dataset (data not shown). In contrast to the apparently low fungal sensitivity, the invertebrates that ingest mycelia exposed to pesticides generally show much more depressed activity and survival. This phenomenon is exacerbated by compensatory mechanisms when shredders increase their consumption of leaves due to reduced nutritional quality caused by lower leaf-associated microbial biomass (Rasmussen et al., 2012c). In our streams, however, no depressed biomass of fungal decomposers was observed and a slight stimulation of fungal reproductive activity was even detected, which may indirectly result from depressed grazing by leaf-associated macroinvertebrates. In addition, an influence of modified fungal community structure, i.e. modified nutritional resource for shredders (Gonçalves et al., 2014), cannot be precluded. The higher abundance of *F. curvula*, a highly palatable species, together with the lower abundance of *L. aquatica* and *T. marchalianum*, two unpalatable species, in the most contaminated streams (Table S2) would tend to nutritionally compensate for the unfavourable environment. All these suggest that the deleterious effects on invertebrate decomposers were more related to their direct exposure to pesticides than affected by trophic cascades.

While microbial-driven breakdown was unaffected by our gradient of pesticides, this lack of sensitivity was apparently further supported by the absence of correlation between TUs and both leaf-associated fungal biomass and conidial production rate. Correlation of conidial production rate with both $\mathrm{mTU}_{\mathrm{algae}}$ and $\mathrm{sTU}_{\mathrm{algae}}$ in our study was however close to be significant ($P = 0.077$ and 0.060, respectively), with the positive relationships showing that the reproductive activity of fungi tended to be stimulated by an increased pesticide toxicity to algae. The same stimulatory effect of various toxicants, sometimes at high concentrations, on conidial production rate has been reported in the literature, e.g. with copper (Roussel et al., 2008). This has generally been attributed to the lower competition for substrate and/or lower predation

by invertebrates (Bärlocher, 1980). Both mechanisms may have been involved here. Some herbicides were present at high levels in our streams (e.g. acetochlor, mTU_{algae}: 0.80), most probably inducing a lower development of algae (Morin et al., 2010) and a decreased competition with fungi in their colonization of leaf blades. Besides, the strongly depressed abundance of shredders (together with other functional feeding groups such as scrapers) in our most contaminated streams may have led to a reduced pressure on the development of reproductive structures and conidial release from leaf surfaces. Consistently with this hypothesis, the dramatic reduction in invertebrate abundance led to an increased conidial production by aquatic hyphomycetes in the manipulated stream reaches that received the insecticide methoxychlor (Suberkropp & Wallace, 1992). In microcosm studies, aquatic hyphomycetes even show enhanced growth at low insecticide concentrations, i.e. values however exceeding by several orders of magnitude the concentrations found in the present study. The same stimulation has been reported with other pesticides (e.g. herbicide; Tsui et al., 2001) as well as other toxicants, suggesting that fungi may use them as sources for their metabolism. Nevertheless, it must be stressed that these stimulatory effects were unlikely on fungal biomass, whereas they probably affected reproductive activity, and they did not translate into enhanced microbial-driven breakdown rate.

In their review, Peters et al. (2013) concluded that the reduction in leaf breakdown due to toxicants was generally more pronounced in studies when invertebrate occurred compared to those where microorganisms were the only decomposers. The imbalance between leaf litter decomposers was markedly evident in our streams where the abatement in invertebrates' involvement along the pesticide gradient was not paralleled by a drop in microbial contribution. The loss of invertebrates' contribution was not compensated by an increased activity of microorganisms. It must be emphasized that leaf-associated bacterial activity was not assessed in our study and differential responses of bacteria and fungi, which may eventually result in stable microbial-driven breakdown, cannot be ruled out. Bacterial leaf decomposers are generally reported to occur at late decomposition stages (Baldy et al., 2007), and thus the interference of bacteria with fungi or other decomposers was unlikely and bacterial contribution to leaf processing probably marginal during our experimental period. As a consequence of the unbalanced decomposers response, $k_{total}/k_{microbe}$ and $k_{invertebrate}/k_{microbe}$ ratios were found to be particularly appropriate to describe the functional impairment. This was also supported by the very high correlation between these ratios and TUs in our streams. Detecting changes in relative contribution of decomposers in ecosystem function even in cases of apparently unaltered rate (i.e. when the activities of decomposer types are compensated each other) strongly argue for the application of such metrics in stream health monitoring.

The effects of pesticides on leaf decomposers and breakdown rates were similarly pronounced when toxicity was expressed in cumulative (sTU) and peak (mTU) terms. Consistently with previous findings (e.g. Von der Ohe et al., 2009), this suggests that one pesticide drove most of the TUs even though the preeminently toxic pesticides differed among our streams (data not shown). Overall this may imply a simply additive effect of the various pesticides, as supported by results on fungicide/insecticide combined effect on leaf-associated fungi and shredder (Flores et al., 2014). Again, using proper tests involving leaf-associated fungal decomposers, especially when considering both direct and indirect effects to higher trophic level, i.e. shredders (Zubrod et al., 2015), may lead to a more realistic picture of fungicide toxicity. Interestingly, $SPEAR_{pesticide}$ was also strongly related to pesticide contamination (Liess & Von der Ohe, 2005) with the identification to family level being sufficient to detect impact (Beketov et al., 2013), which gives credit to insights into the functional structure of macroinvertebrate assemblages with regard to toxic effects. Breakdown rates and $SPEAR_{pesticide}$ were, however, not correlated as in Schäfer et al. (2007). This lack of correlation was due to a single site, Bergon (S10), which exhibited diversified macroinvertebrate assemblages on leaf litter (Table S3), despite its heavy contamination resulting in the second highest value of $SPEAR_{pesticide}$ in our dataset. Moreover, the consideration of invertebrates associated with decomposing leaves in our study, and not the whole invertebrate community presumably more representative of the exposure to pesticides (Schäfer et al., 2007), may explain the (slight) discrepancy between both studies. Indeed, while both breakdown rates and $SPEAR_{pesticide}$ calculated from litterbags responded to pesticide

contamination measured in our sites, this finding should be taken with caution since both metrics can be influenced by confounding factors like morphological habitat degradation (Bunzel et al., 2013) or other anthropogenic disturbances (Hagen et al., 2006; Englert et al., 2015). It remains that the correlation of both metrics with pesticide contamination strongly suggests that pesticides were the main stressors in our agricultural streams. In particular, ammonium, which was potentially suspected to contribute to toxicity towards invertebrates in farmland streams was not correlated with decreased breakdown rates, although concentrations at some sites [e.g. Sausse (S8)] could have been deleterious to leaf-shredding invertebrate taxa (Lecerf et al., 2006). Unlike the $SPEAR_{pesticide}$ index, a stressor-specific metric that has been demonstrated to be invaluable in situations of pesticide contamination, leaf breakdown rates respond to a wide range of chemical stressors including pesticides and other contaminants, point and diffuse pollutions as well as hydromorphological, biological and other environmental disturbances (Webster & Benfield, 1986; Gessner & Chauvet, 2002). In contrast to isolated studies on single compounds and target organisms, which fail to reproduce realistic situations, such a functional approach based on litter breakdown, an integrative ecosystem-process involving a variety of non-target organisms at different trophic levels, is particularly appropriate to evaluate ecologically relevant impacts, i.e. immediate and long-term effects of low concentrations of chemical mixtures usually present in the natural environment. In addition, we found that the impairment of the ecosystem functioning occurred along a range of pesticide concentrations all below the threshold defined by the European Union as the EC_{50} of *Daphnia* divided by an assessment factor of 100 (EEC, 1991; cf. arrow in Fig. 1), confirming that such an approach is not sufficiently sensitive to reveal ecosystem dysfunctioning (Peters et al., 2013). Our work has stressed the importance of disentangling microbial- and invertebrate-driven breakdown rates as ratios of total to microbial-driven breakdown rates were here the most pertinent metric to detect the ecosystem functioning impairment. Future research should evaluate whether this metric is similarly discriminant when using other leaf species and for other stressors, together with the relevance of other ecosystem processes, possibly involving other non-target organisms, to assess the effect of pesticide contaminations.

Acknowledgments We thank two anonymous reviewers for their helpful comments. We are very grateful to Didier Lambrigot and Robert Fernandez for ergosterol determination and field assistance and to Margaux Saüt and coworkers from the Adour-Garonne Water Agency for providing water chemistry data. This work was financially supported by the CIFRE PhD Plan of the French Association for Research and Technology, the Adour-Garonne Water Agency, Asconit Consultants and the Centre National de la Recherche Scientifique (CNRS).

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