Environmental Toxicology and Chemistry—Volume 42, Number 9—pp. 2007–2018, 2023
Received: 26 September 2022 | Revised: 29 November 2022 | Accepted: 25 January 2023

Environmental Toxicology

Standard Versus Natural: Assessing the Impact of Environmental Variables on Organic Matter Decomposition in Streams Using Three Substrates

Verena C. Schreiner,[a,*] Liana Liebmann,[b,c] Alexander Feckler,[a,d] Matthias Liess,[b,e] Moritz Link,[a] Anke Schneeweiss,[a] Amélie Truchy,[f,g] Wolf von Tümpling,[b] Philipp Vormeier,[b,e] Oliver Weisner,[a,b] Ralf B. Schäfer,[a] and Mirco Bundschuh[a,f]

[a]iES Landau, Institute for Environmental Sciences, RPTU Kaiserslautern-Landau, Landau, Germany
[b]Helmholtz Centre for Environmental Research, UFZ, Leipzig, Germany
[c]Department Evolutionary Ecology & Environmental Toxicology, Faculty of Biological Sciences, Institute of Ecology, Diversity and Evolution, Goethe University Frankfurt, Frankfurt am Main, Germany
[d]Eusserthal Ecosystem Research Station, RPTU Kaisterslautern-Landau, Eusserthal, Germany
[e]Institute for Environmental Research, RWTH Aachen University, Aachen, Germany
[f]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden
[g]INRAE, Centre Lyon-Grenoble Auvergne-Rhône-Alpes, Villeurbanne, France

Abstract: The decomposition of allochthonous organic matter, such as leaves, is a crucial ecosystem process in low-order streams. Microbial communities, including fungi and bacteria, colonize allochthonous organic material, break up large molecules, and increase the nutritional value for macroinvertebrates. Environmental variables are known to affect microbial as well as macroinvertebrate communities and alter their ability to decompose organic matter. Studying the relationship between environmental variables and decomposition has mainly been realized using leaves, with the drawbacks of differing substrate composition and consequently between-study variability. To overcome these drawbacks, artificial substrates have been developed, serving as standardizable surrogates. In the present study, we compared microbial and total decomposition of leaves with the standardized substrates of decotabs and, only for microbial decomposition, of cotton strips, across 70 stream sites in a Germany-wide study. Furthermore, we identified the most influential environmental variables for the decomposition of each substrate from a range of 26 variables, including pesticide toxicity, concentrations of nutrients, and trace elements, using stability selection. The microbial as well as total decomposition of the standardized substrates (i.e., cotton strips and decotabs) were weak or not associated with that of the natural substrate (i.e., leaves, $r^2 < 0.01$ to $r^2 = 0.04$). The decomposition of the two standardized substrates, however, showed a moderate association ($r^2 = 0.21$), which is probably driven by their similar composition, with both being made of cellulose. Different environmental variables were identified as the most influential for each of the substrates and the directions of these relationships contrasted between the substrates. Our results imply that these standardized substrates are unsuitable surrogates when investigating the decomposition of allochthonous organic matter in streams. *Environ Toxicol Chem* 2023;42:2007–2018. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Leaf decomposition; Decotabs; Cotton strips; Agriculture; Fungicides; Insecticides; Stressors

INTRODUCTION

The decomposition of allochthonous organic matter is a crucial ecosystem process in freshwater systems, especially in low-order streams with forested catchments where primary production contributes much less to the system's productivity (Graça & Canhoto, 2006; Vannote et al., 1980). After entering streams, allochthonous organic material, such as leaves and wood, is initially colonized by fungi and bacteria (Bärlocher, 2005;

This article includes online-only Supporting Information.

* Address correspondence to schreiner-verena@rptu.de or verena.c.schreiner@gmail.com
Published online 31 January 2023 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5577

Pascoal & Cássio, 2004). These microorganisms break up large molecules such as lignin and cellulose, and build up microbial biomass, thereby increasing protein and lipid contents and thus the nutritional value of the organic matter for shredding macroinvertebrates (Bärlocher, 1985; Gessner et al., 1999; Jenkins & Suberkropp, 1995). Ultimately, the organic matter is transformed into small (<1 mm) particles, representing a large proportion of fine particulate organic matter, which in turn is an important food source for deposit feeders and collectors (Gessner et al., 1999; Vannote et al., 1980).

The distribution of freshwater (micro)organisms is controlled by a wide range of environmental variables. Anthropogenic activities leading to changes in these variables often result in community shifts, which may affect the decomposition of allochthonous organic matter, with consequences for the entire stream food web (Bundschuh et al., 2021; Ferreira et al., 2016; Gomes et al., 2018; Piggott et al., 2015). Moderate nutrient elevation (Gulis & Suberkropp, 2003; Robinson & Gessner, 2000; Truchy et al., 2022) or higher flow velocity (Benfield et al., 2001) can stimulate decomposition. In contrast, pesticides, especially fungicides and insecticides, can harm microbial as well as macroinvertebrate communities and reduce decomposition (Fernández et al., 2015; Rasmussen, Wiberg-Larsen, et al., 2012; Schäfer et al., 2012). Similarly, trace elements, such as copper with its toxic potential for microorganisms, can reduce the decomposition of allochthonous organic matter by altering microbial decomposer communities and their activity (Duarte et al., 2008; Flemming & Trevors, 1989; Zubrod et al., 2015).

Studying the relationship between organic matter decomposition (OMD) and environmental variables in streams has a long history (e.g., Jenkins & Suberkropp, 1995) in which most studies used leaves from local tree species as substrate (Feckler & Bundschuh, 2020). This may result in between-study variability because the decomposition of different leaf species can greatly differ due to the varying chemical composition including nitrogen (N), phosphorous (P), and lignin contents (Bruder et al., 2014; Lecerf & Chauvet, 2008; Zhou et al., 2020). Similarly, the same leaf species from different geographic origins may exhibit variability in its chemical composition, although this is typically much lower than for different leaf species (Bruder et al., 2014; Fernandes et al., 2013; Santschi et al., 2018). Moreover, the collection, quality sorting, storing, and handling of leaves are time- and labour-intensive and standardized procedures are lacking, which may cause variation in decomposition measurements. For example, leaves may be air-dried or frozen, with frozen leaves exhibiting higher initial leaching and consequently higher mass loss during deployment in streams (Bärlocher, 1992) or deployed in bags of different material, mesh size, and construction, which in turn might alter the accessibility of leaves. To overcome these drawbacks, various artificial substrates have been developed with the aim of using them as standardized surrogates, with the benefits of being cheaper and easier to handle (Ferreira et al., 2020). These standardized substrates include decotabs, usually made of agarose and celluloses (Hunting et al., 2016; Kampfraath et al., 2012; Van der Lee et al., 2020), wooden popsicle sticks, and cotton strips, with the latter two only being suitable to quantify microbial decomposition (Maharning & Bärlocher, 1996; Tiegs et al., 2013). Until today, however, the decomposition of cotton strips and leaves was only directly compared at sites exhibiting similar environmental conditions or under controlled conditions, that is, in mesocosms (Tiegs et al., 2007; Truchy et al., 2020), whereas to our knowledge, the decomposition of decotabs was compared with the decomposition of neither leaves nor cotton strips. Thus, it remains unknown how the decompositions of different substrates relate to each other at sites along a gradient of environmental conditions including anthropogenic stressors and whether the decomposition of different substrates responds similarly to this gradient. This knowledge, however, is crucial to justify the use of such standardized substrates as surrogates for investigating ecosystem processes such as OMD.

The aim of the present study was to determine the sole microbially and the combined (i.e., microbially and macroinvertebrate-mediated) decomposition (further referred to as microbial and total decomposition, respectively) of natural and artificial substrates. The substrates included black alder leaves, decotabs, and cotton strips, which were deployed at 70 stream sites in a Germany-wide study. To our knowledge, this is the first study comparing the decomposition of leaves (a natural substrate) and two standardized substrates across sites with varying environmental conditions, in the present study mainly associated with agricultural land use. From a wide range of environmental variables, including anthropogenic stressors such as pesticide exposure and nutrient enrichment, we identified the variables best explaining the decomposition of each of the substrates using a stability selection approach. We hypothesized that the most influential environmental variables, those showing the strongest relationship to OMD, differed between the assessed substrates because the substrates are likely colonized by different microbial communities with varying susceptibility to stressors (cf., Gulis, 2001; Thomas et al., 1992). Nevertheless, we expected congruent responses in the decomposition of the most influential environmental variables, expressed in the similar directions of the relationships between single environmental variables and decomposition.

MATERIALS AND METHODS

General study design

To capture the peak in pesticide application and the related in-stream exposure, the study was conducted in June and July 2019 at 70 stream sites (Supporting Information, Figure S1) distributed over Germany as part of a Germany-wide monitoring campaign (Kleingewässermonitoring; Liess et al., 2021a). Thus, the study period preceded the natural input of leaf litter during autumn and the results might differ from those of studies conducted in autumn. Details on site selection are provided in Liess et al. (2021a). Briefly, most ($n = 59$) of the sites were located in small (mostly <30 km² catchment size, on average 17.6 km² catchment size, second to fourth Strahler order) agricultural streams (average agricultural land cover 75% in the upstream catchment), whereas 11 sites were only

marginally influenced by agricultural land use (<20% agricultural land use in the catchment).

Microbial decomposition was determined using leaves, decotabs, and cotton strips as substrates (see details below). Substrates were separately sewed into fine mesh bags (mesh size 500 μm) and deployed in triplicate at each sampling site by mounting them on stainless-steel bars. Deployment (duration 16–26 days) took place from June 10 to June 14, 2019, while retrieval took place between July 1 and July 9, 2019.

Total decomposition was only determined with leaves and decotabs because macroinvertebrates do not typically feed on cotton strips. To allow feeding by macroinvertebrates, substrates were separately sewed into coarse mesh bags (mesh size 7 mm) and triplicates were mounted at each sampling site on stainless-steel bars. While decotabs were deployed simultaneously with the substrates used for the determination of microbial decomposition, leaves were deployed for a shorter duration (6–8 days) between June 3 and June 7, 2019, and June 10 and June 14, 2019. The shorter deployment duration of the leaves was motivated by the high decomposition rate observed during earlier studies, ensuring an accurate determination of total decompositions (personal observation, after 2 weeks ~50% of leaf bags were retrieved empty).

Additional bags ($n = 8$–12 per substrate and decomposition type) containing one of the three substrates were taken along all process steps, including transportation to the field, to correct for handling losses and to determine the initial ash-free dry mass (AFDM) of the leaves as well as of the decotabs and the tensile strength of cotton strips. The measured decomposition was normalized for the deployment duration and the average temperature at the stream sites (details below).

Substrate handling and processing: leaves

Black alder (*Alnus glutinosa* (L.) Gaertn.) leaves were collected shortly before senescence in 2018. For the determination of microbial decomposition, leaves were collected in a biosphere reserve (at 49.24 N, 7.89 E) close to Landau, South-West Germany. Leaves for the total decomposition were collected adjacent to the Parthe stream (51.21 N, 12.70 E) close to Leipzig, Middle-East Germany. Subsequently, all leaves were quality sorted (i.e., leaves with traces of herbivory and infections were removed) and leaf stems were removed. Afterwards, leaves were air-dried and stored in the dark at room temperature until use. Fine and coarse mesh bags were filled with 8 ± 0.05 and 6 ± 0.05 g of leaves and then deployed for 16–26 and 6–8 days, respectively.

At the end of the deployment, the leaf bags were gently washed in the stream to remove sediment as well as organisms (for the coarse mesh bags, a few remaining organisms were removed manually) and transported cooled (at ~4 °C) to the laboratory, where they were stored at −20 °C. The OMD was estimated based on AFDM to correct for the remaining inorganic substrate. The AFDM was determined after burning the leaves to ash for 5 h at 500 °C and relating the mass to the same dried (60 °C for 48 h) sample. The OMD (in %) was calculated per degree day to normalize for site temperature and deployment duration as follows:

$$\mathrm{OMD} = \frac{\left(\frac{\mathrm{AFDM}_{t0} - \mathrm{AFDM}_{t1}}{\mathrm{AFDM}_{t0}}\right) \times 100\%}{Tn}$$

where AFDM_{t0} and AFDM_{t1} are AFDM before (normalized for handling losses) and after deployment, respectively, T is the average temperature over the deployment period, and n refers to the number of days of deployment (summed up as degree days). Determining the total OMD and not the macroinvertebrate-specific OMD resulted in a high remaining number of replicates with positive values and thus enabled a direct comparison between the total OMD of leaves and decotabs.

Because of the loss of bags, drying of streams, lack of reliable temperature data, or complete decomposition of the substrate on retrieval, we present data from 64 and 66 out of 70 sites for microbial and total leaf decomposition, respectively.

Substrate handling and processing: decotabs

Decotabs with a diameter of 35 mm and 8 mm in height were prepared following Kampfraath et al. (2012). Briefly, 150 ml of ultrapure water with 3 g of agarose was heated using a microwave until the agarose dissolved, cooled to 50 °C, and 9 g of powdered cellulose was added. The mixture was subsequently poured into acrylic glass moulds and put into a fridge (4 °C) to solidify the tabs. If the decotabs were still convex after cooling down, excess material was removed with a surgical blade. The decotabs were prepared approximately 2 weeks before the deployment, individually sewn into coarse and fine mesh bags, and stored as well as transported at 4 °C. Their OMD was determined as described for leaves (see section *Substrate handling and processing: leaves*).

Due to loss of bags, drying of streams, lack of reliable temperature data, or complete decomposition (the case for 54% of the decotabs in coarse mesh bags) of the substrate on retrieval, we were able to use decotabs data from 61 and 34 out of 70 sites for microbial and total decotabs decomposition, respectively.

Substrate handling and processing: cotton strips

Cotton strips were cut from artists' fabric in the dimensions 8×2.5 cm following Tiegs et al. (2013). Before sewing them into mesh bags, the cotton strips were pre-leached in ultrapure water for 48 h to remove any chemicals potentially remaining from the manufacturing process and dried at 60 °C. At the end of the deployment, the surface of the strips was cleaned from attached material and stored frozen (−20 °C). Before determining the tension loss—the proxy for microbial OMD—the cotton strips were dipped in ethanol, dried at 60 °C for 24 h, and the tensile strength was determined using a tensiometer (Mark-10 brand, Model ESM303). The peak tension necessary to tear the cotton strips apart was recorded per strip. The

tensile strength loss (in % per degree day) was calculated similarly to the OMD, with $AFDM_{t1}$ and $AFDM_{t0}$ being replaced by the peak tension of the exposed and the average peak tension of the handling control cotton strips, respectively.

Due to loss of bags, drying of streams, lack of temperature data, or incorrect tearing of the cotton strips (e.g., tearing right below, above or in between the grips of the tensiometer), we were able to use cotton strips data from 63 out of 70 sites.

Environmental variables and local macroinvertebrate community

During the Kleingewässermonitoring project (between April and July 2019; Liess et al., 2021a), a wide range of environmental variables were measured following the scheme described in this section. We restricted our analysis to average values of measurements during the two deployment periods of the analyzed substrates (Table 1). Every 3 weeks, pH and temperature (single measurements, continuous measurement see below) were determined using a pH meter (WTW Multi 3630 IDS Set G or Greisinger G 1500) and the flow velocity was measured using either a flow meter (Höntzsch) or via a drifting unit (Schwoerbel, 1995). At the same time, grab water samples were taken in the middle of the each stream. In addition, during heavy rainfall causing a water level rise, water samples were taken using an automated event-driven sampler (MAXX TP5). The procedure is detailed in Liess et al. (2021a) and Neale et al. (2020). These grab and event water samples were used to measure several environmental variables. First, concentrations of ortho-phosphate, nitrate, nitrite, and ammonium were

TABLE 1: Overview of environmental variables characterizing the sampling sites during the deployment period of substrates

Variable	$n \pm SD$[a]	Unit	Analysis	Min.	Max.	Median	Mean	SD
Stream type[b]		NA	Both	2.1	19	7	10	5.6
Catchment size[c]		km²	Both	1.6	270	11	16	33
Proportion of agricultural land use in upstream catchment		%	Both	0	100	63	55	32
Fine substrate (<2 mm)		%	Both	0	100	30	41	28
Coarse particulate organic matter[c]		%	Total	0	70	0	7	13
Dissolved oxygen[d]	Probes[e]	mg L^{-1}	Both	1.8	9.6	7.6	7	1.9
Stream temperature during deployment[d,f]	Probes	°C		10	21	17	16	2
pH[d]	2 ± 0.41	NA	Both	5.9	8.8	7.7	7.6	0.52
Conductivity[d]	Probes	µS cm^{-1}	Both	94	1800	550	600	360
Flow velocity[d]	1.3 ± 0.47	m s^{-1}	Both	0	0.8	0.23	0.24	0.16
sumTU for fungi[d]	2.9 ± 0.72	NA	Both	−4.79	−1.53	−2.44	−2.51	0.53
sumTU for invertebrates[d]	2.9 ± 0.72	NA	Total	−6.13	−0.54	−3.14	−3.15	1.3
Phosphate[c,d]	2.6 ± 0.81	mg L^{-1}	Both	0.07	6	0.46	0.8	1
Nitrate[d]	2.6 ± 0.81	mg L^{-1}		1.3	81	9.1	15	15
Nitrite[d]	2.4 ± 0.87	mg L^{-1}	Both	0.03	1	0.14	0.21	0.2
Ammonium[c,d]	2.4 ± 0.88	mg L^{-1}	Both	0.01	1	0.1	0.21	0.25
Total phosphorus[c,d]	2.5 ± 0.81	mg L^{-1}	Both	0	1.2	0.08	0.12	0.17
Total nitrogen[c,d]	2.5 ± 0.81	mg L^{-1}	Both	0.16	28	3.3	4.9	4.7
Arsenic[c,d]	2.5 ± 0.77	mg L^{-1}	Both	0.05	7.3	1	1.4	1.5
Cadmium[d]	2.5 ± 0.77	mg L^{-1}		0.005	0.25	0.01	0.02	0.03
Copper[d]	2.5 ± 0.77	mg L^{-1}	Both	0.1	6.1	2.1	2.4	1.5
Mercury[c,d]	2.5 ± 0.77	mg L^{-1}	Both	0.001	0.04	0.003	0.005	0.01
Lead[d]	2.5 ± 0.77	mg L^{-1}		0.03	2.2	0.03	0.1	0.27
Zinc[c,d]	2.5 ± 0.77	mg L^{-1}	Both	0.5	17	2	2.5	2.8
$SPEAR_{pesticides}$		NA	Total	0	1	0.47	0.48	0.2
Abundance shredder per m²[g]		NA		0.24	9600	130	530	1600
Proportion shredder		%	Total	0.03	67	28	28	17

[a]The number of samples varied per sampling site due to different numbers of rainfall events and single failures of measuring devices. During rainfall events, in case of incomplete sampling (caused by short high water level durations) the water samples were prioritized as follows: (i) pesticide analysis, (ii) nutrient analysis, and (iii) trace element analysis.
[b]This variable refers to the biocoenotic stream type (Pottgiesser & Sommerhäuser, 2004).
[c]These variables were log-transformed (decadic logarithm) to alleviate skewness.
[d]These variables were either recorded repeatedly (i.e., in continuous intervals complemented by event-driven samples) during the deployment period or via probes and in Table 1 we present the average per site.
[e]Measurements via probes were conducted every 15 or 30 min.
[f]The stream temperature during deployment was used to normalize the decomposed organic mass (details see section *Substrate handling and processing: leaves*).
[g]The abundance for the feeding group shredders was calculated based on the fuzzy codes of this trait. This means that a taxon was not necessarily only categorized as a shredder.
In Table 1, we present the average value of environmental variables during the deployment of microbial decomposition substrates and the total decomposition of decotabs (see Supporting Information, Table S1, for the range of environmental variables of the total decomposition of leaves). In addition, we present the number of samples (*n*) and their standard deviation (SD) for variables where we calculated average values. Variables indicated with "Both" are used for statistical analysis of microbial and total decomposition, whereas variables indicated with "Total" are only used for the analysis of total decomposition. For nutrients and trace elements, values below the limit of quantification (LOQ) were set to half of the LOQ, whereas values above the qualification range were set to twice the potential highest value.

measured without pre-processing steps on-site using a UV spectrophotometer (DR 1900; Hach Lange) and the corresponding cuvette tests (Hach Lange). Second, concentrations of total P and total N as well as trace elements (arsenic, cadmium, copper, zinc, lead, and mercury) were analyzed via inductively coupled plasma mass spectrometry (ICP-MS 8800 Triple Quad; Agilent). Samples for the analysis of arsenic, cadmium, copper, zinc, and lead were filtered on-site (0.45 µm syringe filter), while the samples to analyze mercury concentrations were stabilized using a nitric acid and potassium dichromate solution and stored at 4 °C until analysis. Finally, the concentrations of 76 pesticides were analyzed using an LC-MS/MS (Agilent 1290 infinity liquid chromatography system coupled to a QTrap6500+ tandem mass spectrometer equipped with an electrospray ionization [ESI] interface; Sciex). Details on the pesticide analysis are provided by Halbach et al. (2021).

To estimate the potential toxicity toward fungi and invertebrates, either the 25 detected fungicides or all detected pesticides were considered when calculating the logarithmic sum of toxic units (sumTU) as follows:

$$\text{sumTU} = \log\left(\sum \frac{c_i}{\text{EC}_{50i}}\right)$$

where c is the concentration of pesticide i and EC50_i is the concentration of pesticide i at which 50% of the test organisms were affected. When calculating the sumTU for fungi (hereafter $\text{sumTU}_{\text{fungi}}$), we selected the respective lowest EC50 value of either the most sensitive freshwater invertebrate or algae because EC50 values for fungi are lacking and we anticipated that fungi react more sensitively to fungicides than invertebrates or algae (Ittner et al., 2018). For the sumTU for invertebrates (hereafter $\text{sumTU}_{\text{inv}}$), we selected the respective lowest EC50 value of the most sensitive freshwater invertebrate, which proved to be the most responsive measure in a comparative analysis (Schäfer et al., 2013). The EC50 values were collected via the R-package Standartox (Scharmüller et al., 2020) and complemented by values from Lewis et al. (2016).

In addition to tri-weekly measurements, temperature (continuous measurements), oxygen concentrations, and conductivity were constantly measured using multiparameter probes (LogTrans7-compact measuring system SENSOdive CTDO2, UIT equipped with O_2-Log3055-INT and CTD3100–10 loggers; Driesen + Kern). Due to the failure of a few probes or too low water levels (10 and nine streams for the two deployment periods, respectively), values were missing for individual streams. Missing average temperatures during substrate deployment were complemented by estimations based on single measurements and logger data of neighbouring sites. Because the normalization of decomposition for cumulative temperature (degree day over deployment period) can vary strongly, only temperatures estimated using information from streams with similar temperature regimes were used for the analysis. This resulted in missing temperature information for five and four streams for the two deployment periods (see above), respectively.

By using the R-package "openSTARS" (Kattwinkel & Szöcs, 2022), a digital elevation model (cell size 25 × 25 m) and CORINE land cover data (European Environment Agency, 2018), we derived information on the catchment size and the proportion of agricultural land use in the upstream catchment (aggregated CORINE classes 2.11, 2.21 and 2.22).

At the beginning of June 2019, macroinvertebrates were sampled using standardized multihabitat sampling over approximately 50-m long stream stretches (Meier et al., 2006). The ratio and abundance of shredders were calculated based on trait data retrieved from freshwaterecology.info (Schmidt-Kloiber & Hering, 2015). The $\text{SPEAR}_{\text{pesticides}}$, a metric to describe the sensitivity of macroinvertebrate communities toward pesticides, was calculated as described in Liess et al. (2021a). During the multihabitat sampling, the biocoenotic stream type (Pottgiesser & Sommerhäuser, 2004), as well as the ratios of fine substrate (smaller than 2 mm) and coarse particulate organic (CPOM) matter were recorded.

Data analysis

To compare the microbial decomposition of the three substrates (i.e., leaves, decotabs, and cotton strips) and the total OMD of two substrates (i.e., leaves and decotabs), we conducted three and one Pearson's correlations, respectively. The inter-replicate variability (%) was calculated as the variability in OMD of one set of replicates divided by its mean.

For identifying the environmental variables most influential for the decomposition, we used average values for the environmental variables measured consecutively during the respective deployment periods. Before calculating the averages, all nutrient and trace element concentrations below the limit of quantification (LOQ) were set to half of the LOQ, while values above the qualification range were set to twice the potential highest value. For variables continuously measured via probes (conductivity and dissolved oxygen), we calculated median values (Table 1). From the environmental variables, we omitted those from further analysis (i.e., nitrate and abundance of shredders) that exhibited strong intercorrelation (Pearson's correlation > 0.7) and variance inflation factors above five (Table 1; Lin, 2008). Furthermore, we removed variables with a low range or a high number of values below LOQ (i.e., cadmium and lead). Variables that exhibited a strong skewness were log-transformed (Table 1).

The variables most influential for the decomposition of the different substrates were identified using stability selection (Meinshausen & Bühlmann, 2010). This method estimates the probability that a variable is selected in a model via bootstrapping and, in comparison with cross-validation methods, reduces the number of falsely selected variables. Using the package "stabs" (Hofner & Hothorn, 2021), we selected a cutoff value of 0.7 (i.e., a variable was selected in 70% of the fitted models) and a per-family error rate of 1 (i.e., a maximum of one variable is tolerated to be falsely selected per model). Thus, variables that were selected with a higher frequency can be considered more relevant. Before running the analysis, the explanatory variables were standardized to an average of 0 and a standard deviation of 1 to remove the effect of different ranges and magnitudes. We displayed the relationship of the

substrates' decomposition to the respective selected environmental variables using marginal effects plots (Lüdecke, 2018).

Data preparation, statistical analysis, and visualizations were conducted in R (Ver 4.2.1; R Core Team, 2022) using additional packages including "ggplot2" (Wickham, 2016; full list of all packages and their versions is given in the R code). We provide raw data on decomposition, processed environmental variables, and the computer code under https://github.com/VCSchr/Standard_vs_Natural. The raw data of the environmental variables are provided by Liess et al. (2021b) at https://doi.org/10.1594/PANGAEA.931673.

RESULTS

Microbial decomposition

The OMD of decotabs (median 0.06% per degree day, mean 0.08%, SD 0.05; absolute decomposed substrate mass mean 58.6%, SD 28.9%) was the lowest, followed by the OMD of leaves (median 0.14% per degree day, mean 0.14%, SD 0.04; absolute decomposed substrate mass mean 24.5%, SD 32.8%) and the tension loss of cotton strips (median 0.19% per degree day, mean 0.18%, SD 0.06; absolute tension loss mean 47.4%, SD 12.7%; Figure 1). The inter-replicate variability of the microbially decomposed substrates was with 0.46% (absolute value = 0.0007) the lowest for leaves, whereas it was with 0.92% and 1.5% a factor of two to three higher for cotton strips and decotabs respectively (absolute values 0.0014 and 0.0015, respectively). The OMD of leaves was not or only weakly associated with that of the two standardized substrates, decotabs, and cotton strips ($r^2 < 0.01$, $p = 0.898$ and $r^2 = 0.03$ $p = 0.053$, respectively; Figure 1A,B). The decomposition of the standardized substrates showed a moderate correlation ($r^2 = 0.21$, $p < 0.001$; Figure 1C), which means that approximately 20% of the variance of one substrate could be explained by the other substrate.

Total decomposition

The total OMD of decotabs had a median of 0.16% (mean 0.15% per degree day, SD 0.07; absolute decomposed substrate mass mean 49.8%, SD 24.8%) lower than the OMD of leaves with 0.40% (mean 0.45% per degree day, SD 0.19;

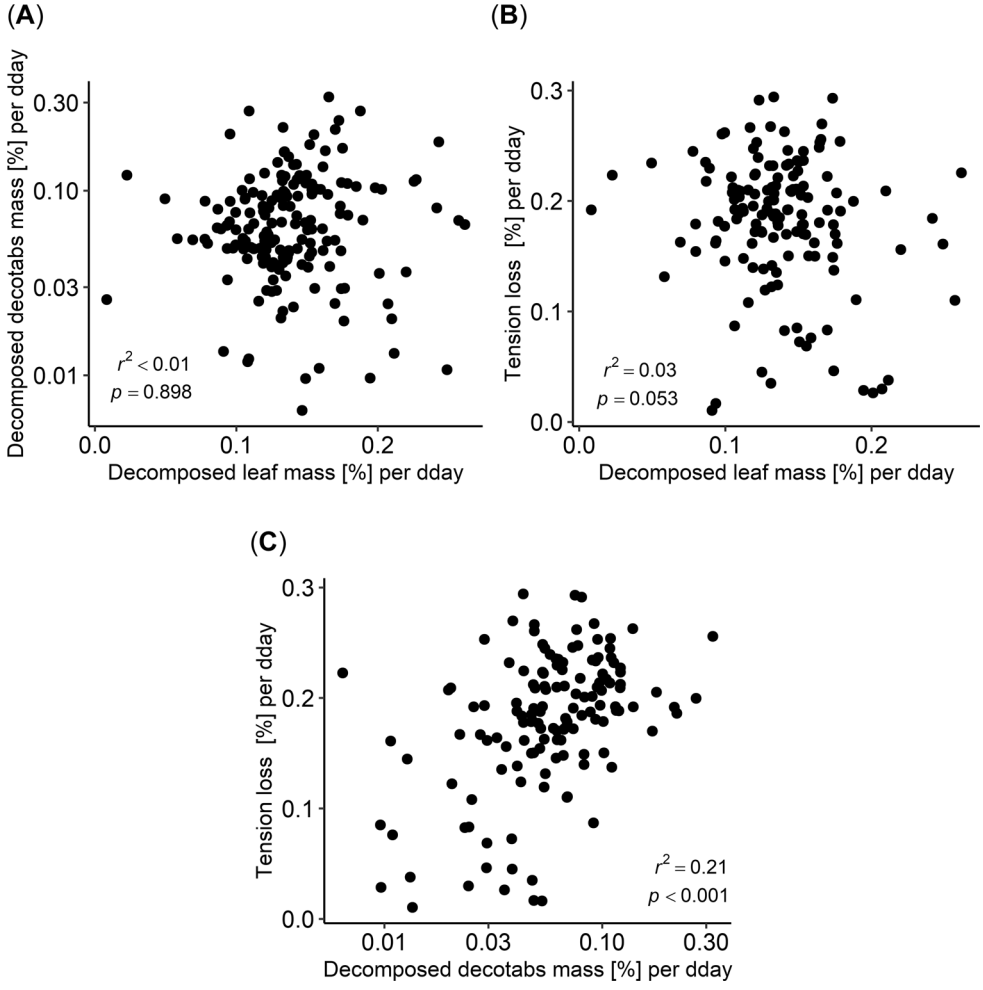

FIGURE 1: Relationships between the microbial decomposition of different substrates. The decomposition of all substrates was normalized for deployment duration and temperature (degree day, dday). (**A**) Decomposed organic mass of decotabs versus decomposed organic mass of leaves. (**B**) Tension loss of cotton strips versus decomposed organic mass of leaves. (**C**) Tension loss of cotton strips versus decomposed organic mass of decotabs. The decomposed organic mass of decotabs is displayed on a logarithmic scale (see Supporting Information, Figure S2, for nonlogarithmic scaled decomposition of decotabs).

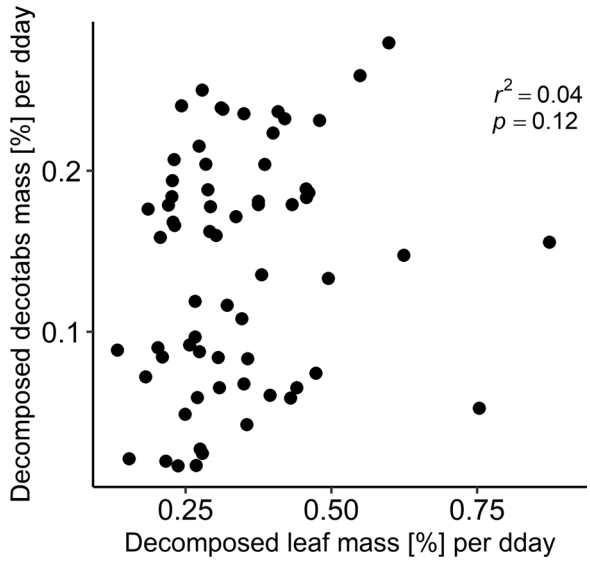

FIGURE 2: Relationship between the total decomposition of leaves and decotabs. The decomposition of the substrates was normalized for deployment duration and temperature (degree day, dday).

absolute decomposed substrate mass mean 51.6%, SD 21.3%). The OMD for decotabs, however, was likely underestimated because 54% of the coarse mesh bags were retrieved empty and thus removed from the analysis. The inter-replicate variability of total OMD was with 1.73% (absolute 0.008) higher for leaves than for decotabs (relative = 0.74%, absolute = 0.0009). The total OMD of leaves was weakly related to the OMD of decotabs ($r^2 = 0.10$, $p = 0.118$; Figure 2).

Variables explaining microbial and total decomposition

None of the environmental variables identified in the statistical analysis was most influential to the microbial and total decomposition of all substrates (Table 2). Furthermore, the majority of variables showed contrasting relationships to the decomposition of the different substrates, with only four (dissolved oxygen, pH, logarithmic total P concentration, and estimated toxicity toward fungi [sumTU$_{fungi}$]) of 26 environmental variables exhibiting the same direction.

Variables explaining microbial decomposition

The most influential environmental variables for microbial decomposition differed among the three substrates (i.e., leaves, cotton strips, and decotabs; Table 2 and Supporting Information, Figure S3). The OMD of leaves was strongest and positively related to flow velocity (Supporting Information, Figure S3A), followed by negative relationships with the logarithmic arsenic concentration and the proportion of agricultural land use in the catchment, that is, the general agricultural impact (Supporting Information, Figure S3B,C). In contrast to the OMD of leaves, the tension loss of cotton strips was positively related to the proportion of agricultural land use in the catchment (Supporting Information, Figure S3D). The OMD of decotabs was positively related to the estimated toxicity toward fungi (sumTU$_{fungi}$; Supporting Information, Figure S3E).

TABLE 2: Selection frequency of the environmental variables explaining the respective decomposition of the substrates

	Microbial						Total			
	Leaves		Cotton strips		Decotabs		Leaves		Decotabs	
Stream type	–	n.s.	+	0.02	+	0.01	–	0.15	o	0.02
Catchment size (log)	o	0.04	+	0.09	o	0.08	–	0.49	+	0.10
Proportion of agriculture	**–**	**0.76**	**+**	**0.85**	(+)	0.02	–	0.26	+	0.17
Fine substrate	(–)	0.01	(–)	0.02	+	0.19	+	0.03	–	0.18
Dissolved oxygen	+	n.s.	+	n.s.	+	n.s.	+	n.s.	+	n.s.
pH	o	0.01	–	0.04	–	0.09	–	0.08	–	0.22
Conductivity	(+)	n.s.	–	n.s.	–	n.s.	–	n.s.	–	n.s.
Flow velocity	**+**	**0.98**	–	0.01	–	0.02	–	0.01	+	0.04
sumTU fungicides	+	n.s.	+	0.25	**+**	**0.78**	+	0.03	o	0.35
Phosphate (log)	+	0.01	–	0.11	–	n.s.	o	n.s.	–	0.05
Nitrite[a]	(–)	n.s.	–	0.01	o	0.14	+	n.s.	–	0.15
Ammonium (log)	o	0.01	o	0.36	–	n.s.	–	n.s.	+	0.19
Total phosphorus (log)	o	0.01	+	0.43	+	0.28	+	0.22	**+**	**0.86**
Total nitrogen (log)	(–)	n.s.	+	0.51	+	0.14	+	0.56	–	0.01
Arsenic (log)[a]	**–**	**0.80**	+	0.04	+	0.63	–	0.06	–	n.s.
Copper	–	0.09	–	n.s.	o	0.03	+	0.06	–	0.01
Mercury (log)	(+)	0.11	(–)	0.02	o	0.08	–	0.06	o	0.56
Zinc (log)	+	0.05	–	n.s.	–	0.26	–	0.26	–	0.01
Ratio coarse particulate organic matter (log)		n.i.		n.i.		n.i.	+	0.09	o	0.08
sumTU invertebrates		n.i.		n.i.		n.i.	o	0.05	**+**	**0.70**
Shredder proportion		n.i.		n.i.		n.i.	+	0.40	+	0.06
SPEAR$_{pesticides}$		n.i.		n.i.		n.i.	**+**	**0.85**	o	0.02

[a]Nitrite was log-transformed and arsenic was not log-transformed when identifying the most relevant environmental variables for the total decomposition.
Selected variables (i.e., above the cut-off value of 0.7 in the stability selection) are marked in bold. n.s. = variable was not selected in stability section; n.i. = not included in analysis. The direction of the relationships is indicated by: + = positive relationship, – = negative relationship; o = no tendency. Symbols in brackets refer to weak relationships.

Variables explaining total decomposition

The total decomposition of the two substrates (i.e., leaves and decotabs) is best explained by different variables (Table 2 and Supporting Information, Figure S3). Although the OMD of leaves was positively related to the estimated pesticide impact on the macroinvertebrate community (i.e., $SPEAR_{pesticides}$; Supporting Information, Figure S3F), the OMD of decotabs was positively related to the logarithmic total phosphorus concentration and the estimated toxicity toward invertebrates ($sumTU_{inv}$; Supporting Information, Figure S3G,H).

DISCUSSION

Microbial decomposition

For microbial decomposition, we detected a weak or no relationship between the standardized substrates (i.e., cotton strips and decotabs) and the natural substrate (i.e., black alder leaves; Figure 1), which suggests that standardized substrates are not ideal surrogates for natural substrates to assess microbial OMD in streams. Nevertheless, the positive weak relationship between the microbial OMD of leaves and the tension loss of cotton strips (Figure 1) detected in our study is in agreement with Tiegs et al. (2007). The correlation in this earlier study was, however, stronger, which may be explained by different experimental designs, including the use of different leaf species (black alder in the present study vs. black poplar [*Populus nigra*] in Tiegs et al. [2007]). Moreover, our study covered sites with varying environmental conditions whereas Tiegs et al. (2007) assessed the decomposition over different habitats within one stream section with comparable environmental conditions.

The lack of strong correlations between leaves and standardized substrates may be explained by both standardized substrates mainly consisting of cellulose (Boulton & Quinn, 2000; Hunting et al., 2016; Imberger et al., 2010), therefore they represent a simplified substrate in terms of complexity and are likely to be colonized by a more specialized and thus functionally limited microbial community in comparison with black alder leaves (Gulis, 2001; Thomas et al., 1992). Leaves in comparison are composed of a more diverse set of organic polymers, including lignin (Bruder et al., 2014; Lecerf & Chauvet, 2008; Zhou et al., 2020). Due to their chemical composition, the standardized substrates lack central nutrients such as P and N (Imberger et al., 2010; Tiegs et al., 2013, 2019), which likely makes the associated microbial communities highly dependent on external nutrient sources. The access to these may be uneven at different stream locations driven by hydromorphological conditions (e.g., higher flow velocities make higher amounts of resources accessible; Burgazzi et al., 2020). Consequently, the dependency on external nutrients, whose availability was likely highly variable in our study, and the lack of buffer capacity by microbial communities may result in higher inter-replicate variability of the standardized relative to the natural substrates. The low inter-replicate variability of the natural substrate in the present study can be further explained by a standardized selection procedure of leaves for use in the study likely leading to a rather homogenous chemical composition among replicates.

Total decomposition

Similar to the microbial decomposition, the total OMD of leaves and decotabs showed a weak relationship, which in turn demonstrates that decotabs are not ideal surrogates for natural substrates to assess total and likely also net macroinvertebrate OMD. The total OMD of leaves was higher than that of the decotabs. However, more than half of the bags containing decotabs at deployment were retrieved empty (54%) and thus excluded from further analyses and interpretation, underestimating the OMD of decotabs. Nevertheless, the higher OMD of leaves might result from a feeding preference of shredders, a macroinvertebrate feeding group that is highly selective when it comes to food (Arsuffi & Suberkropp, 1989; Graça et al., 2001). This selectivity is driven by substrate properties and likely the microbial communities colonizing these (Gulis, 2001; Thomas et al., 1992), as discussed above. Furthermore, the inter-replicate variability of leaves was clearly higher than that of decotabs, which could be related to the very short deployment duration of leaves in comparison with decotabs (6–8 vs. 16–26 days). This short deployment period likely favoured a patchy colonization by the aquatic microbial community, probably resulting in a more variable feeding source for shredders (Pascoal & Cássio, 2004).

Environmental variables explaining decomposition

We were able to confirm our hypothesis that the most influential environmental variables for decomposition differed between substrates (Table 2). In contrast to our expectations, however, the different substrates showed incongruent responses to the same environmental variables because the directions of the relationships differed (Table 2). Yet, because the standardized substrates, that is, decotabs and cotton strips, capture only a rather limited proportion of the entire leaf decomposition process (cellulose vs. more complex composition of organic polymers) varying responses to environmental variables are plausible. This observation consequently suggests that standardized substrates are not suitable representatives of their natural counterpart when quantifying microbial and total OMD. Using standardized substrates may, in fact, be misleading when analysing the effects of environmental variables on ecosystem functioning (in our case the decomposition of allochthonous material) and, thereby, ultimately misinform management.

None of the environmental variables identified in the statistical analysis was most influential to all substrates. Only one variable, the proportion of agricultural land use in the upstream catchment, was identified as most influential for two substrates (i.e., leaves and cotton strips, both for microbial decomposition). The directions of these relationships were, however, contrasting (Table 2 and Supporting Information, Figure S3C,D): while a

negative impact of agriculture on the OMD of leaves is in line with previous studies (Liess et al., 2022; Rasmussen, Wiberg-Larsen, et al., 2012), the tension loss of cotton strips was positively related to this variable, a trend reported in few studies using leaves and focussing on microbial respiration (Griffiths et al., 2009; Rossi et al., 2019).

Only four of 26 analysed environmental variables exhibited the same direction of the relationship for the decomposition of all substrates. Among these was the estimated toxicity toward fungi ($sumTU_{fungi}$), which was positively related to all substrates targeting microbial decomposition, as well as the leaves for total decomposition. Overall, the $sumTU_{fungi}$ was the single most influential variable on the microbial OMD of decotabs (Table 2 and Supporting Information, Figure S3E). Previous studies, however, found a negative impact of fungicides and antibiotics on fungal communities and the microbial OMD of leaves across different seasons (Fernández et al., 2015; Rasmussen, Monberg, et al., 2012; Schreiner et al., 2018) as well as on OMD of decotabs (Kampfraath et al., 2012). An explanation for the positive relationship could be a community turnover resulting in the prevalence of tolerant taxa having a higher decomposition capacity compared with unaltered communities (Baudy et al., 2021; Feckler et al., 2018; Schreiner et al., 2018). Although we lack data to support this assumption from the present study, the detected $sumTU_{fungi}$ corresponded to a range where strong fungal community turnovers were observed elsewhere (Fernández et al., 2015; Schreiner et al., 2018). Another environmental variable exhibiting the same direction of the relationship with the decomposition of most substrates was the logarithmic total P concentration. The logarithmic total P concentration showed a positive relationship to the decomposition of substrates used for macroinvertebrate as well as the microbial decomposition of cotton strips and decotabs (Table 2 and Supporting Information, Figure S3G). For the total OMD of decotabs, this environmental variable was selected as one of the most influential, which is in agreement with previous studies (e.g., Gulis & Suberkropp, 2003; Robinson & Gessner, 2000; Truchy et al., 2022). Moreover, the data partially support the assumption discussed above, namely that the lack of nutrients within the substrate increases dependency on external sources (Imberger et al., 2010; Tiegs et al., 2013, 2019).

Interestingly, two variables which captured the pesticide toxicity toward macroinvertebrate communities showed positive relationships with total OMD for leaves and decotabs, respectively. The total OMD of leaves showed a positive relationship to the potential recent pesticide impact on the macroinvertebrate community ($SPEAR_{pesticides}$), which is in concert with previous studies (Schäfer et al., 2007, 2012). The positive relationship between the total OMD of decotabs and the estimated toxicity toward invertebrates ($sumTU_{inv}$) is in contrast to previous studies, which detected a negative relationship with decomposition (Magali et al., 2016; Schäfer et al., 2012). Other studies reported ambiguous relationships between the estimated toxicity toward invertebrates and decomposition (Link et al., 2022; Rasmussen, Wiberg-Larsen, et al., 2012), which may be explained by differing gradient lengths. The fact that only the total OMD of decotabs but not of leaves exhibited a relationship to $sumTU_{inv}$ may be explained by different toxicity levels and related gradients during the two deployment periods (Table 1 and Supporting Information, Table S1). During leaf deployment, the estimated toxicity toward invertebrates was, based on the median, higher than during deployment of decotabs (median $sumTU_{inv}$ of −2.58 vs. −3.14) though during both deployment periods the estimated maximal toxicity was similar (maximum $sumTU_{inv}$ of −0.65 and −0.54, respectively).

CONCLUSIONS

The decomposition of the standardized substrates, decotabs and cotton strips, showed weak or no relationships to the decomposition of the natural substrate consisting of black alder leaves, for microbial as well as for total decomposition in streams. This suggests that standardized substrates are unreliable surrogates to investigate the ecosystem process of OMD. This statement is backed up by the fact that these standardized substrates mainly consist of cellulose, ignoring other components of natural substrates such as leaves, which will attract more diverse microbial and likely also macroinvertebrate communities. The ability of microorganisms to take up nutrients from the surrounding water could buffer for low nutrient availability from the substrate and thereby supporting their functional performance (Costello et al., 2022). Furthermore, the composition of decotabs could be adjusted to resemble the chemical composition of natural substrates such as leaves by adding further ingredients (Hunting et al., 2016; Vonk et al., 2016). In other words, a real-world situation can hardly be reproduced using these rather simplistic standardized substrates, a criticism well documented in terrestrial (Howard, 1988) and aquatic (Tiegs et al., 2013) studies. The difference among substrates used in the present study likely drives the varying responses in their decomposition toward changes in environmental variables, therefore, when using standardized substrates, results might be of minor relevance for decomposition processes in natural systems and estimates of the relevance of single environmental variables are likely biased. However, differences in chemical composition or deployment times may also be relevant among other natural substrates, including different leaf species or other dead plant parts. This suggests additional efforts are needed before final conclusions about the suitability of artificial, standardized substrates as surrogates for natural substrates can be drawn.

Supporting Information—The Supporting Information is available on the Wiley Online Library, at https://doi.org/10.1002/etc.5577.

Acknowledgments—The project and Verena C. Schreiner were funded by the Deutsche Forschungsgemeinschaft (DFG—Project number 216374258). Furthermore, this project was part of the pilot study on the monitoring of small streams (Kleingewässermonitoring) implemented by the UFZ-Helmholtz-Centre for Environmental Research and the German Federal Ministry for the Environment, Nature Conservation and Nuclear

Safety (FKZ 3717 63 403 0). We thank the students who supported the laboratory and field work, and T. Reemtsma of the Department of Analytical Chemistry from the Helmholtz Centre for Environmental Research (UFZ), Germany, for the pesticide analysis. Open Access funding enabled and organized by Projekt DEAL.

Author Contributions Statement—**Verena C. Schreiner**: Conceptualization; Methodology; Formal analysis; Investigation; Writing—original draft; Writing—review & editing; Visualization. **Liana Liebmann**: Formal analysis; Investigation; Writing—review & editing. **Alexander Feckler**: Investigation; Writing—review & editing. **Matthias Liess**: Writing—review & editing; Project administration; Funding acquisition. **Moritz Link**: Investigation; Writing—review & editing. **Anke Schneeweiss**: Investigation; Writing—review & editing. **Amélie Truchy**: Investigation; Writing—review & editing. **Wolf von Tümpling**: Investigation; Writing—review & editing. **Philipp Vormeier**: Investigation; Writing—review & editing. **Oliver Weisner**: Investigation; Writing—review & editing. **Ralf B. Schäfer**: Conceptualization; Writing—review & editing; Project administration; Funding acquisition. **Mirco Bundschuh**: Conceptualization; Writing—review & editing.

Data Availability Statement—We provide raw data on decomposition, processed environmental variables, and the computer code under https://github.com/VCSchr/Standard_vs_Natural. The raw data for the environmental variables are provided at https://doi.org/10.1594/PANGAEA.931673.

REFERENCES

Arsuffi, T. L., & Suberkropp, K. (1989). Selective feeding by shredders on leaf-colonizing stream fungi: Comparison of macroinvertebrate taxa. *Oecologia*, *79*(1), 30–37. https://doi.org/10.1007/BF00378236

Bärlocher, F. (1985). The role of fungi in the nutrition of stream invertebrates. *Botanical Journal of the Linnean Society*, *91*(1–2), 83–94. https://doi.org/10.1111/j.1095-8339.1985.tb01137.x

Bärlocher, F. (1992). Effects of drying and freezing autumn leaves on leaching and colonization by aquatic hyphomycetes. *Freshwater Biology*, *28*(1), 1–7. https://doi.org/10.1111/j.1365-2427.1992.tb00556.x

Bärlocher, F. (2005). Freshwater fungal communities. In J. Dighton & J. F. White (Eds.), *The fungal community: Its organization and role in the ecosystem* (3rd ed., pp. 39–60). CRC Press.

Baudy, P., Zubrod, J. P., Konschak, M., Röder, N., Nguyen, T. H., Schreiner, V. C., Baschien, C., Schulz, R., & Bundschuh, M. (2021). Environmentally relevant fungicide levels modify fungal community composition and interactions but not functioning. *Environmental Pollution*, *285*, 117234. https://doi.org/10.1016/j.envpol.2021.117234

Benfield, E. F., Webster, J. R., Tank, J. L., & Hutchens, J. J. (2001). Long-term patterns in leaf breakdown in streams in response to watershed logging. *International Review of Hydrobiology*, *86*(4–5), 467–474. https://doi.org/10.1002/1522-2632(200107)86:4/5%3C467::AID-IROH467%3E3.0.CO;2-1

Boulton, A. J., & Quinn, J. M. (2000). A simple and versatile technique for assessing cellulose decomposition potential in floodplain and riverine sediments. *Archiv Für Hydrobiologie*, *150*(1), 133–151. https://doi.org/10.1127/archiv-hydrobiol/150/2000/133

Bruder, A., Schindler, M. H., Moretti, M. S., & Gessner, M. O. (2014). Litter decomposition in a temperate and a tropical stream: The effects of species mixing, litter quality and shredders. *Freshwater Biology*, *59*(3), 438–449. https://doi.org/10.1111/fwb.12276

Bundschuh, M., Feckler, A., Schäfer, R. B., Schulz, R., & Zubrod, J. P. (2021). How toxicants influence organic matter decomposition in streams. In C. M. Swan, L. Boyero, & C. Canhoto (Eds.), *The ecology of plant litter decomposition in stream ecosystems* (pp. 379–410). Springer International Publishing. https://doi.org/10.1007/978-3-030-72854-0_17

Burgazzi, G., Laini, A., Ovaskainen, O., Saccò, M., Stubbington, R., & Viaroli, P. (2020). Communities in high definition: Spatial and environmental factors shape the micro-distribution of aquatic invertebrates. *Freshwater Biology*, *65*(12), 2053–2065. https://doi.org/10.1111/fwb.13599

Costello, D. M., Tiegs, S. D., Boyero, L., Canhoto, C., Capps, K. A., Danger, M., Frost, P. C., Gessner, M. O., Griffiths, N. A., Halvorson, H. M., Kuehn, K. A., Marcarelli, A. M., Royer, T. V., Mathie, D. M., Albariño, R. J., Arango, C. P., Aroviita, J., Baxter, C. V., Bellinger, B. J., & Zwart, J. A. (2022). Global Patterns and Controls of Nutrient Immobilization on Decomposing Cellulose in Riverine Ecosystems. *Global Biogeochemical Cycles*, *36*, e2021GB007163. https://doi.org/10.1029/2021GB007163

Duarte, S., Pascoal, C., Alves, A., Correia, A., & Cássio, F. (2008). Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. *Freshwater Biology*, *53*(1), 91–101. https://doi.org/10.1111/j.1365-2427.2007.01869.x

European Environment Agency. (2018). *CLC 2018—Copernicus Land Monitoring Service 2018*. European Environment Agency (EEA). https://land.copernicus.eu/pan-european/corine-land-cover/clc2018

Feckler, A., & Bundschuh, M. (2020). Decoupled structure and function of leaf-associated microorganisms under anthropogenic pressure: Potential hurdles for environmental monitoring. *Freshwater Science*, *39*(4), 652–664. https://doi.org/10.1086/709726

Feckler, A., Goedkoop, W., Konschak, M., Bundschuh, R., Kenngott, K. G. J., Schulz, R., Zubrod, J. P., & Bundschuh, M. (2018). History matters: Heterotrophic microbial community structure and function adapt to multiple stressors. *Global Change Biology*, *24*(2), e402–e415. https://doi.org/10.1111/gcb.13859

Fernandes, I., Duarte, S., Cássio, F., & Pascoal, C. (2013). Effects of riparian plant diversity loss on aquatic microbial decomposers become more pronounced with increasing time. *Microbial Ecology*, *66*(4), 763–772. https://doi.org/10.1007/s00248-013-0278-0

Fernández, D., Voss, K., Bundschuh, M., Zubrod, J. P., & Schäfer, R. B. (2015). Effects of fungicides on decomposer communities and litter decomposition in vineyard streams. *Science of the Total Environment*, *533*, 40–48. https://doi.org/10.1016/j.scitotenv.2015.06.090

Ferreira, V., Elosegi, A. D., Tiegs, S., von Schiller, D., & Young, R. (2020). Organic matter decomposition and ecosystem metabolism as tools to assess the functional integrity of streams and rivers—A systematic review. *Water*, *12*(12), 12. https://doi.org/10.3390/w12123523

Ferreira, V., Koricheva, J., Duarte, S., Niyogi, D. K., & Guérold, F. (2016). Effects of anthropogenic heavy metal contamination on litter decomposition in streams—A meta-analysis. *Environmental Pollution*, *210*, 261–270. https://doi.org/10.1016/j.envpol.2015.12.060

Flemming, C. A., & Trevors, J. T. (1989). Copper toxicity and chemistry in the environment: A review. *Water, Air, and Soil Pollution*, *44*(1–2), 143–158. https://doi.org/10.1007/BF00228784

Gessner, M. O., Chauvet, E., & Dobson, M. (1999). A perspective on leaf litter breakdown in streams. *Oikos*, *85*(2), 377–384. https://doi.org/10.2307/3546505

Gomes, P. P., Ferreira, V., Tonin, A. M., Medeiros, A. O., & Júnior, J. F. G. (2018). Combined effects of dissolved nutrients and oxygen on plant litter decomposition and associated fungal communities. *Microbial Ecology*, *75*(4), 854–862. https://doi.org/10.1007/s00248-017-1099-3

Graça, C. ressa, Gessner, F. eio, & Callies, B. arrios (2001). Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology*, *46*(7), 947–957. https://doi.org/10.1046/j.1365-2427.2001.00729.x

Graça, M. A., & Canhoto, C. (2006). Leaf litter processing in low order streams. *Limnetica*, *25*(1–2), 1–10.

Griffiths, N. A., Tank, J. L., Royer, T. V., Rosi-Marshall, E. J., Whiles, M. R., Chambers, C. P., Frauendorf, T. C., & Evans-White, M. A. (2009). Rapid decomposition of maize detritus in agricultural headwater streams. *Ecological Applications*, *19*(1), 133–142. https://doi.org/10.1890/07-1876.1

Gulis, V. (2001). Are there any substrate preferences in aquatic hyphomycetes? *Mycological Research*, *105*(9), 1088–1093. https://doi.org/10.1017/S0953756201004439

Gulis, V., & Suberkropp, K. (2003). Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. *Aquatic Microbial Ecology*, *30*(2), 149–157. https://doi.org/10.3354/ame030149

Halbach, K., Möder, M., Schrader, S., Liebmann, L., Schäfer, R. B., Schneeweiss, A., Schreiner, V. C., Vormeier, P., Weisner, O., Liess, M., & Reemtsma, T. (2021). Small streams–large concentrations? Pesticide monitoring in small agricultural streams in Germany during dry weather and rainfall. *Water Research*, *203*, 117535. https://doi.org/10.1016/j.watres.2021.117535

Hofner, B., & Hothorn, T. (2021). stabs: Stability selection with error control (0.6-4). https://CRAN.R-project.org/package=stabs

Howard, P. J. A. (1988). A critical evaluation of the cotton strip assay. In A. F. Harrison, P. M. Latter, & D. W. H. Walton (Eds.), *Cotton strip assay: An index of decomposition in soils* (pp. 34–42). NERC/ITE.

Hunting, E. R., Vonk, J. A., Musters, C. J. M., Kraak, M. H. S., & Vijver, M. G. (2016). Effects of agricultural practices on organic matter degradation in ditches. *Scientific Reports*, *6*(1), 21474. https://doi.org/10.1038/srep21474

Imberger, S. J., Thompson, R. M., & Grace, M. R. (2010). Searching for effective indicators of ecosystem function in urban streams: Assessing cellulose decomposition potential. *Freshwater Biology*, *55*(10), 2089–2106. https://doi.org/10.1111/j.1365-2427.2010.02469.x

Ittner, L. D., Junghans, M., & Werner, I. (2018). Aquatic fungi: A disregarded trophic level in ecological risk assessment of organic fungicides. *Frontiers in Environmental Science*, *6*, 105. https://doi.org/10.3389/fenvs.2018.00105

Jenkins, C. C., & Suberkropp, K. (1995). The influence of water chemistry on the enzymatic degradation of leaves in streams. *Freshwater Biology*, *33*(2), 245–253. https://doi.org/10.1111/j.1365-2427.1995.tb01165.x

Kampfraath, A. A., Hunting, E. R., Mulder, C., Breure, A. M., Gessner, M. O., Kraak, M. H. S., & Admiraal, W. (2012). DECOTAB: A multipurpose standard substrate to assess effects of litter quality on microbial decomposition and invertebrate consumption. *Freshwater Science*, *31*(4), 1156–1162. https://doi.org/10.1899/12-075.1

Kattwinkel, M., & Szöcs, E. (2022). openSTARS: An Open Source Implementation of the 'ArcGIS' Toolbox 'STARS' (1.2.3). https://CRAN.R-project.org/package=openSTARS

Lecerf, A., & Chauvet, E. (2008). Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic and Applied Ecology*, *9*(5), 598–605. https://doi.org/10.1016/j.baae.2007.11.003

Lewis, K. A., Tzilivakis, J., Warner, D. J., & Green, A. (2016). An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal*, *22*(4), 1050–1064.

Liess, M., Liebmann, L., Lück, M., Vormeier, P., Weisner, O., Foit, K., Knillmann, S., Schäfer, R. B., Schulze, T., Krauss, M., Brack, W., Reemtsma, T., Halbach, K., Link, M., Schreiner, V. C., Schneeweiss, A., Möder, M., Weitere, M., Kaske, O., & Borchardt, D. (2022). *Umsetzung des Nationalen Aktionsplans zur nachhaltigen Anwendung von Pflanzenschutzmitteln (NAP)—Pilotstudie zur Ermittlung der Belastung von Kleingewässern in der Agrarlandschaft mit Pflanzenschutzmittel-Rückständen*. Umweltbundesamt. https://www.umweltbundesamt.de/publikationen/umsetzung-des-nationalen-aktionsplans-zur-1

Liess, M., Liebmann, L., Vormeier, P., Weisner, O., Altenburger, R., Borchardt, D., Brack, W., Chatzinotas, A., Escher, B., Foit, K., Gunold, R., Henz, S., Hitzfeld, K. L., Schmitt-Jansen, M., Kamjunke, N., Kaske, O., Knillmann, S., Krauss, M., Küster, E., & Reemtsma, T. (2021a). Pesticides are the dominant stressors for vulnerable insects in lowland streams. *Water Research*, *201*, 117262. https://doi.org/10.1016/j.watres.2021.117262

Liess, M., Liebmann, L., Vormeier, P., Weisner, O., Altenburger, R., Borchardt, D., Brack, W., Chatzinotas, A., Escher, B., Foit, K., Gunold, R., Henz, S., Hitzfeld, K. L., Schmitt-Jansen, M., Kamjunke, N., Kaske, O., Knillmann, S., Krauss, M., Küster, E., & Reemtsma, T. (2021b). The lowland stream monitoring dataset (KgM, Kleingewässer-Monitoring) 2018, 2019 [Data set]. PANGAEA. https://doi.org/10.1594/PANGAEA.931673

Lin, F.-J. (2008). Solving multicollinearity in the process of fitting regression model using the nested estimate procedure. *Quality & Quantity*, *42*(3), 417–426. https://doi.org/10.1007/s11135-006-9055-1

Link, M., Schreiner, V. C., Graf, N., Szöcs, E., Bundschuh, M., Battes, K. P., Cîmpean, M., Sures, B., Grabner, D., Buse, J., & Schäfer, R. B. (2022). Pesticide effects on macroinvertebrates and leaf litter decomposition in areas with traditional agriculture. *Science of the Total Environment*, *828*, 154549. https://doi.org/10.1016/j.scitotenv.2022.154549

Lüdecke, D. (2018). ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software*, *3*(26), 772. https://doi.org/10.21105/joss.00772

Magali, B., Sylvain, L., & Eric, C. (2016). Litter breakdown for ecosystem integrity assessment also applies to streams affected by pesticides. *Hydrobiologia*, *773*(1), 87–102. https://doi.org/10.1007/s10750-016-2681-2

Maharning, A. R., & Bärlocher, F. (1996). Growth and reproduction in aquatic hyphomycetes. *Mycologia*, *88*(1), 80–88. https://doi.org/10.2307/3760786

Meier, C., Haase, P., Rolauffs, P., Schindehütte, K., Schöll, F., Sundermann, A., & Hering, D. (2006). *Methodisches Handbuch Fließgewässerbewertung—Handbuch zur Untersuchung und Bewertung von Fließgewässern auf der Basis des Makrozoobenthos vor dem Hintergrund der EG-Wasserrahmenrichtlinie*. Fliessgewässerbewertung.De. https://gewaesserbewertung.de/files/meier_handbuch_mzb_2006.pdf

Meinshausen, N., & Bühlmann, P. (2010). Stability selection. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *72*(4), 417–473. https://doi.org/10.1111/j.1467-9868.2010.00740.x

Neale, P. A., Braun, G., Brack, W., Carmona, E., Gunold, R., König, M., Krauss, M., Liebmann, L., Liess, M., Link, M., Schäfer, R. B., Schlichting, R., Schreiner, V. C., Schulze, T., Vormeier, P., Weisner, O., & Escher, B. I. (2020). Assessing the mixture effects in in vitro bioassays of chemicals occurring in small agricultural streams during rain events. *Environmental Science & Technology*, *54*(13), 8280–8290. https://doi.org/10.1021/acs.est.0c02235

Pascoal, C., & Cássio, F. (2004). Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Applied and Environmental Microbiology*, *70*(9), 5266–5273. https://doi.org/10.1128/AEM.70.9.5266-5273.2004

Piggott, J. J., Niyogi, D. K., Townsend, C. R., & Matthaei, C. D. (2015). Multiple stressors and stream ecosystem functioning: Climate warming and agricultural stressors interact to affect processing of organic matter. *Journal of Applied Ecology*, *52*(5), 1126–1134. https://doi.org/10.1111/1365-2664.12480

Pottgiesser, T., & Sommerhäuser, M. (2004). Fließgewässertypologie Deutschlands: Die Gewässertypen und ihre Steckbriefe als Beitrag zur Umsetzung der EU-Wasserrahmenrichtlinie. In C. E. W. Steinberg, C. Calmano, R.-D. Wilken, & H. Klapper (Eds.), *Handbuch der Limnologie* (pp. 1–61). John Wiley & Sons, Ltd. https://doi.org/10.1002/9783527678488.hbal2004005

R Core Team. (2022). R: A language and environment for statistical computing. http://www.R-project.org/

Rasmussen, J. J., Monberg, R. J., Baattrup-Pedersen, A., Cedergreen, N., Wiberg-Larsen, P., Strobel, B., & Kronvang, B. (2012). Effects of a triazole fungicide and a pyrethroid insecticide on the decomposition of leaves in the presence or absence of macroinvertebrate shredders. *Aquatic Toxicology*, *118–119*, 54–61. https://doi.org/10.1016/j.aquatox.2012.03.015

Rasmussen, J. J., Wiberg-Larsen, P., Baattrup-Pedersen, A., Monberg, R. J., & Kronvang, B. (2012). Impacts of pesticides and natural stressors on leaf litter decomposition in agricultural streams. *Science of the Total Environment*, *416*, 148–155. https://doi.org/10.1016/j.scitotenv.2011.11.057

Robinson, C. T., & Gessner, M. O. (2000). Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia*, *122*(2), 258–263. https://doi.org/10.1007/PL00008854

Rossi, F., Mallet, C., Portelli, C., Donnadieu, F., Bonnemoy, F., & Artigas, J. (2019). Stimulation or inhibition: Leaf microbial decomposition in streams subjected to complex chemical contamination. *Science of the Total Environment*, *648*, 1371–1383. https://doi.org/10.1016/j.scitotenv.2018.08.197

Santschi, F., Gounand, I., Harvey, E., & Altermatt, F. (2018). Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. *Functional Ecology*, *32*(2), 522–532. https://doi.org/10.1111/1365-2435.12980

Schäfer, R. B., Bundschuh, M., Rouch, D. A., Szöcs, E., von der Ohe, P. C., Pettigrove, V., Schulz, R., Nugegoda, D., & Kefford, B. J. (2012). Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. *Science of the Total Environment*, *415*, 69–78. https://doi.org/10.1016/j.scitotenv.2011.05.063

Schäfer, R. B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., & Liess, M. (2007). Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. *Science of the Total Environment*, *382*(2–3), 272–285. https://doi.org/10.1016/j.scitotenv.2007.04.040

Schäfer, R. B., Gerner, N., Kefford, B. J., Rasmussen, J. J., Beketov, M. A., de Zwart, D., Liess, M., & von der Ohe, P. C. (2013). How to characterize chemical exposure to predict ecologic effects on aquatic communities.

Environmental Science & Technology, *47*(14), 7996–8004. https://doi.org/10.1021/es4014954

Schäfer, R. B., von der Ohe, P. C., Rasmussen, J., Kefford, B. J., Beketov, M. A., Schulz, R., & Liess, M. (2012). Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environmental Science & Technology*, *46*(9), 5134–5142. https://doi.org/10.1021/es2039882

Scharmüller, A., Schreiner, V. C., & Schäfer, R. B. (2020). Standartox: Standardizing toxicity data. *Data*, *5*(2), 46. https://doi.org/10.3390/data5020046

Schmidt-Kloiber, A., & Hering, D. (2015). An online tool that unifies, standardises and codifies more than 20 000 European freshwater organisms and their ecological preferences. *Ecological Indicators*, *53*, 271–282. https://doi.org/10.1016/j.ecolind.2015.02.007

Schreiner, V. C., Feckler, A., Fernández, D., Frisch, K., Muñoz, K., Szöcs, E., Zubrod, J. P., Bundschuh, M., Rasmussen, J. J., Kefford, B. J., Axelsen, J., Cedergreen, N., & Schäfer, R. B. (2018). Similar recovery time of microbial functions from fungicide stress across biogeographical regions. *Scientific Reports*, *8*(1), 17021. https://doi.org/10.1038/s41598-018-35397-1

Schwoerbel, J. (1995). *Methoden der Hydrobiologie* (4th ed.). Fischer.

Thomas, K., Chilvers, G., & Norris, R. (1992). Aquatic hyphomycetes from different substrates: Substrate preference and seasonal occurrence. *Australian Journal of Marine and Freshwater Research*, *43*(2), 491–509. https://doi.org/10.1071/MF9920491

Tiegs, S. D., Clapcott, J. E., Griffiths, N. A., & Boulton, A. J. (2013). A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecological Indicators*, *32*, 131–139. https://doi.org/10.1016/j.ecolind.2013.03.013

Tiegs, S. D., Costello, D. M., Isken, M. W., Woodward, G., McIntyre, P. B., Gessner, M. O., Chauvet, E., Griffiths, N. A., Flecker, A. S., Acuña, V., Albariño, R., Allen, D. C., Alonso, C., Andino, P., Arango, C., Aroviita, J., Barbosa, M. V. M., Barmuta, L. A., Baxter, C. V., & Zwart, J. A. (2019). Global patterns and drivers of ecosystem functioning in rivers and riparian zones. *Science Advances*, *5*(1), eaav0486. https://doi.org/10.1126/sciadv.aav0486

Tiegs, S. D., Langhans, S. D., Tockner, K., & Gessner, M. O. (2007). Cotton strips as a leaf surrogate to measure decomposition in river floodplain habitats. *Journal of the North American Benthological Society*, *26*(1), 70–77. https://doi.org/10.1899/0887-3593(2007)26[70:CSAALS]2.0.CO;2

Truchy, A., Sarremejane, R., Muotka, T., Mykrä, H., Angeler, D. G., Lehosmaa, K., Huusko, A., Johnson, R. K., Sponseller, R. A., & McKie, B. G. (2020). Habitat patchiness, ecological connectivity and the uneven recovery of boreal stream ecosystems from an experimental drought. *Global Change Biology*, *26*(6), 3455–3472. https://doi.org/10.1111/gcb.15063

Truchy, A., Sponseller, R. A., Ecke, F., Angeler, D. G., Kahlert, M., Bundschuh, M., Johnson, R. K., & McKie, B. G. (2022). Responses of multiple structural and functional indicators along three contrasting disturbance gradients. *Ecological Indicators*, *135*, 108514. https://doi.org/10.1016/j.ecolind.2021.108514

Van der Lee, G. H., Hunting, E. R., Vonk, J. A., & Kraak, M. H. S. (2020). Decomposition and Consumption Tablets (DECOTABs). In F. Bärlocher, M. O. Gessner, & M. A. S. Graça (Eds.), *Methods to study litter decomposition* (pp. 519–525). Springer International Publishing. https://doi.org/10.1007/978-3-030-30515-4_57

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(1), 130–137.

Vonk, J. A., van Kuijk, B. F., van Beusekom, M., Hunting, E. R., & Kraak, M. H. S. (2016). The significance of linoleic acid in food sources for detritivorous benthic invertebrates. *Scientific Reports*, *6*(1), 35785. https://doi.org/10.1038/srep35785

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis [R]. https://ggplot2.tidyverse.org

Zhou, S., Butenschoen, O., Barantal, S., Handa, I. T., Makkonen, M., Vos, V., Aerts, R., Berg, M. P., McKie, B., Van Ruijven, J., Hättenschwiler, S., & Scheu, S. (2020). Decomposition of leaf litter mixtures across biomes: The role of litter identity, diversity and soil fauna. *Journal of Ecology*, *108*(6), 2283–2297. https://doi.org/10.1111/1365-2745.13452

Zubrod, J. P., Feckler, A., Englert, D., Koksharova, N., Rosenfeldt, R. R., Seitz, F., Schulz, R., & Bundschuh, M. (2015). Inorganic fungicides as routinely applied in organic and conventional agriculture can increase palatability but reduce microbial decomposition of leaf litter. *Journal of Applied Ecology*, *52*(2), 310–322. https://doi.org/10.1111/1365-2664.12393

