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Ignite

Environmental context determines pollution impacts on ecosystem functioning

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Global change assessments have typically ignored synthetic chemical pollution, despite the rapid increase of pharmaceuticals, pesticides and industrial chemicals in the environment. Part of the problem reflects the multifarious origins of these micropollutants, which can derive from urban and agricultural sources. Understanding how micropollutants harm ecosystems is a major scientific challenge due to asymmetries of stress across trophic levels and ecological surprises generated by multiple drivers interacting in human-impacted landscapes. We used field assays above and below municipal wastewater treatment plants (WWTPs) in 60 sampling locations across 20 Swiss streams to test how micropollutants and nutrients originating from WWTPs affect two trophic levels (microbes and detritivores) and their role in leaf litter processing. Wastewater impacts were asymmetric across trophic levels, with the detritivore contribution declining relative to microbial-driven decomposition. The strength of negative impacts were context dependent, peaking at sites with the highest upstream abundances of detritivorous invertebrates. Diffuse pollution from intensive agriculture and wastewater-born micropollutants contributed to reduced litter processing rates, including indirect effects apparently mediated through negative influences of insecticides on detritivores. Asymmetries in stress responses across trophic levels can introduce quantitative changes in consumer–resource dynamics and leaf litter processing. This means functional redundancies at different trophic levels are insufficient to compensate for biodiversity losses, causing environmental stressors such as chemical pollutants to have pervasive ecosystem-level impacts.

Keywords: biodiversity, decomposition, micropollutants, multiple stressors, nutrients, wastewater

Introduction

Globally, ecosystems are facing unprecedented rates of change caused by multiple anthropogenic drivers (Harrison et al. 2018, Díaz et al. 2019). At continental scales,



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pollution of aquatic habitats may cause profound changes in biodiversity and ecosystem functioning (Woodward et al. 2012), with synthetic chemicals (e.g. pesticides) posing a pervasive threat (Beketov et al. 2013). Recent studies have further highlighted the dangers posed by specific synthetic chemicals in the environment (Yamamuro et al. 2019, Tian et al. 2021). However, planetary assessments of ecological threats have typically ignored synthetic chemical pollution, despite the rapid increase of these pollutants relative to other drivers of global change, such as eutrophication or carbon emissions (Bernhardt et al. 2017). Predicting ecological consequences of numerous environmental change drivers requires knowing how multiple trophic levels respond to varying levels of perturbation with different stressor combinations (Halstead et al. 2014, Burdon 2020). Asymmetric responses of different trophic levels may be particularly relevant where the same groups of organisms exhibiting sensitivity to stressors (e.g. pesticides) also disproportionately contribute to ecosystem functioning (Stanley et al. 2015, Thompson et al. 2016).

Understanding asymmetric responses to environmental change is essential for predicting indirect effects in ecological networks where trophic interactions help determine community structure and ecosystem functioning (Menge 1995, Tylianakis et al. 2007). We define asymmetric responses as those that differ (i.e. unequal or opposite) in response to the same ultimate driver (e.g. pollution). Consumer–resource interactions are particularly important because they strongly determine how nutrients and materials flux across hierarchical levels of biological organisation (Dell et al. 2014, Burdon 2020). For instance, toxicants can reduce consumer densities, leading to reduced resource depletion rates (Carlisle and Clements 2005, Halstead et al. 2014). In contrast, nutrients can ‘mask’ the negative effects of pesticides on carbon processing, primarily through positive effects on microbial activity (Burdon et al. 2020). Here we sought to understand the causal pathways driving the impacts of anthropogenic pollution on stream ecosystem functioning measured as leaf-litter processing rates.

Leaf-litter processing is a fundamental ecosystem function that influences the global carbon cycle (Boyer et al. 2021b), in addition to food-web dynamics and system performance in a wide range of habitats, including headwater streams (Moore et al. 2004, Tank et al. 2010). In stream ecosystems, litter processing involves both biological and physical factors contributing to mass loss. Decomposition in its strictest sense means all biological processes contributing to organic matter mass loss and transformation, but not including physical losses caused by abrasion, fragmentation or leaching (Gessner et al. 2010). Biological processes contributing to decomposition include microbial conditioning and consumption by detritivores (Hieber and Gessner 2002). Anthropogenic stressors that impact different food-web compartments can also influence decomposition rates, making litter processing assays powerful indicators of ecosystem functioning and environmental change (Gessner and Chauvet 2002).

Detritivore abundances often strongly influence decomposition rates (Hieber and Gessner 2002), but microbes can

play a compensatory role when invertebrates are impacted by pollution (Pascoal et al. 2005). However, the compensatory role of microbial-driven decomposition is often insufficient to fully compensate for the loss of detritivores. For example, chemical pollution that disproportionately harmed detritivorous invertebrates also negatively impacted leaf-litter processing rates (Thompson et al. 2016). Treated effluent from municipal wastewater treatment plants (WWTPs) is a major source of pollution (e.g. nutrient enrichment) in urbanized catchments globally (Hamdhani et al. 2020). WWTPs are the primary contributor of micropollutants (e.g. pharmaceuticals, pesticides and industrial chemicals) to aquatic ecosystems (Schwarzenbach et al. 2006). In particular, pesticides in treated effluent may dominate the toxic pressure on stream invertebrates (Munz et al. 2017, Münze et al. 2017). Studies have highlighted the potential for ecological harm from parasitoids (e.g. veterinary flea treatments containing fiprole and/or imidacloprid) in wastewater (Perkins et al. 2021), and reduced abundances of pesticide-sensitive stream invertebrates downstream of WWTPs have been correlated with slower litter processing rates (Münze et al. 2017).

Pollution of freshwaters has been broadly associated with altered community structure and ecosystem functioning (Woodward et al. 2012). Despite the undeniable impact of these drivers, the underlying mechanisms linking structural changes to ecosystem functioning (e.g. litter processing) have remained contentious. This may be because pollution in aquatic habitats derives from multiple diffuse (e.g. intensive agriculture) and point-sources (e.g. WWTPs) associated with different human activities, meaning negative impacts of synthetic chemicals are often confounded with other stressors (e.g. nutrients) allied with these pressures (Burdon et al. 2019). We previously recorded positive influences of warming and nutrients from WWTPs on microbial activity and cellulose degradation (Burdon et al. 2020). In the present study, we investigated the potential threat that inputs of WWTP effluent and associated synthetic chemicals pose to biodiversity and ecosystem functioning.

Wastewater impacts may be strongly context dependent, with either nutrient-driven positive (Cabrini et al. 2013) or toxicant-driven negative (Münze et al. 2017) effects on litter-processing rates in streams. At low levels of nutrient enrichment, nutrient limitation should constrain decomposition rates, whereas highly-enriched systems may experience stress from co-occurring pollutants thereby negatively affecting detrital consumption (Woodward et al. 2012). Thus, we hypothesised that micropollutants present in wastewater impair the contribution of detritivorous invertebrates to litter processing (Bundschuh et al. 2011), but increased nutrient concentrations and warming associated with inputs of treated effluent would subsidise the microbial decomposition of organic matter (Burdon et al. 2020). Further, we expected that negative wastewater impacts on litter processing would be strongest where upstream abundances of detritivores were greatest (Woodward et al. 2012). Our results demonstrate the threat synthetic chemical pollution poses to aquatic ecosystems and show how functional redundancies across trophic

levels may be unable to combat asymmetric effects of interacting anthropogenic impacts on ecosystems.

Material and methods

Twenty sites in Switzerland were selected to investigate WWTP impacts on receiving stream ecosystems (Burdon et al. 2016, Stamm et al. 2016). Streams had no other WWTPs upstream, treated WW > 20% of total discharge and catchment land-uses < 21% urban and < 10% orcharding by area. At each site, we designated one downstream sampling location (D), and two upstream sampling locations (U1, U2). Sites had comparable stream morphology, riparian land use and vegetation types above and below WWTP. Following Burdon et al. (2019), land use data (Arealstatistik 2009) for site selection and further analysis was obtained from Swiss land use statistics collected 2004–2009 (<www.landuse-stat.admin.ch>). For more details see the Supporting information.

We characterized annual water quality trends at upstream and downstream locations by collecting 936 grab samples. For the 2013 sites, we took monthly grab samples between March 2013 and February 2014. For the 2014 sites, we sampled bi-monthly (from March 2014 to January 2015). We analyzed 20 general water quality parameters at all sampling dates (Supporting information). We analyzed a priority mixture of 57 organic micropollutants (MPs) at two sampling dates (June 2013, February 2014) for the 2013 sites, and MPs and heavy metals (HMs) at all six sampling dates in 2014 (Munz et al. 2017). Toxic units (TUs) were calculated from organic MPs and HMs (Munz et al. 2017). Wastewater quantity is the proportion of treated effluent in the receiving stream (Burdon et al. 2019). Total benthic suspendable sediment (Burdon et al. 2013) from sampling locations were measured in autumn 2013 and 2014. For more details see the Supporting information.

Leaf-litter processing was measured by placing pre-weighed leaf bags in a stream, and then recovering them after a pre-determined time to estimate mass loss (Bärlocher 2020). We used leaves from two European tree species: the common alder *Alnus glutinosa* and the common oak *Quercus robur*. We placed 5 g of dry, recently abscised leaves into leaf-bags using two mesh sizes (fine, 0.5 mm; and coarse, 10 mm) to assess microbial-mediated decomposition (fine mesh) with litter breakdown jointly caused by microbes, invertebrates and physical processes (coarse mesh).

We deployed a total of 1008 leaf bags to our study sites; litter processing assays were deployed in autumn 2013 and 2014. Six leaf bags were replicated per treatment combination at each sampling location (D, U1, U2) with only one sampling date (i.e. time of collection) at 12 sites in 2013 and eight sites in 2014. Only coarse-mesh alder leaf bags were used in 2014. After collection, processing rates (k) with and without temperature correction were calculated. The leaf-litter processing rate coefficient k was first calculated for each leaf bag (Eq. 1):

$$k = \frac{-\ln\left(\frac{W_t}{W_0}\right)}{t} \quad (1)$$

where W_t is the remaining leaf mass, W_0 is the initial leaf mass and t is the incubation period (days). Processing coefficients (k) were also computed using temperature (degree-days). Degree-days were estimated by summing the average daily water temperatures (°C) over each incubation periods, and entering the appropriate values (i.e. the degree-days accumulated from day 1 to the retrieval day) in place of t (Benfield et al. 2017). Both types of rate coefficient were calculated for litter processing in the coarse-mesh bags (k_{total}) and for microbial decomposition in fine-mesh bags ($k_{\text{microbial}}$).

Invertebrate-mediated decomposition ($k_{\text{invertebrate}}$) was also calculated, thereby assessing decomposition attributable to factors other than microbes, primarily detritivore feeding, physical abrasion and fragmentation (McKie et al. 2006). We adapted a new approach for calculating invertebrate-mediated decomposition (including losses due to physical factors) after recognising inconsistencies in Woodward et al. (2012) also highlighted by Lecerf (2017). Firstly, we calculated the mean (\bar{x}) remaining leaf mass in fine mesh leaf-bags $W_{\bar{x}}^F$ from each sampling location using Eq. 2:

$$W_{\bar{x}}^F = \frac{\sum_{k=1}^n W_t^F}{n} \quad (2)$$

where W_t^F is the remaining leaf mass in an individual fine mesh leaf-bag at time t , and n is the number of fine mesh leaf-bags remaining at sampling location. $k_{\text{invertebrate}}$ was then calculated using Eq. 3:

$$k_{\text{invertebrate}} = \frac{-\ln\left(\frac{W_t^C}{W_{\bar{x}}^F}\right)}{t} \quad (3)$$

where W_t^C is the remaining leaf mass in an individual coarse mesh leaf-bag at the end of the incubation period, time t (days or degree-days). When $W_t^C > W_{\bar{x}}^F$ (i.e. the leaf mass remaining in the coarse mesh leaf-bag was greater than the average remaining leaf mass in fine mesh leaf-bags), we assigned $k_{\text{invertebrate}} = 0$. Finally, a dimensionless metric was calculated using the log-transformed ratio of coefficients (Eq. 4) from invertebrate and microbial mediated decomposition in order to quantify and evaluate the relative contribution of microbes and invertebrates to the litter processing (Hladyz et al. 2011):

$$k\text{-ratio} = \ln\left(\frac{k_{\text{invertebrate}}}{k_{\text{microbial}}} + 1\right) \quad (4)$$

Aquatic invertebrates from coarse mesh leaf-bags were identified and enumerated to Family level. Using trait data

(Usseglio-Polatera et al. 2000), community-weighted means (CWM) for the 'food' trait modality 'plant detritus > 1 mm' and the 'feeding habits' trait modality 'shredder' were calculated using the *dbFD* function in FD package in R (Laliberté et al. 2014). The CWM is defined as the mean of values present in the community weighted by the relative abundance of taxa bearing each value (Lavorel et al. 2008). The results of our statistical models using each trait modality were very similar, so only the 'Food' trait modality 'plant detritus > 1 mm' is presented in the main text.

We used linear mixed-effects models (LME) to test wastewater impacts on litter processing rates (*k*/day and *k*/degree-day) and mean daily stream temperature with a fixed effect of location (D, U1, U2) and random effect of *Site* nested in *Year*. For these LME we used data from coarse alder litter bags collected from 60 sampling locations over two years (2013 and 2014). We used LME (random effect: *Site*) to test WW impacts on alder and oak litter processing, invertebrate and microbial-mediated decomposition, the *k*-ratio and mean daily stream temperatures at the 2013 sites. LME were fitted with the *lmer4* (Bates et al. 2015) and *blme* (Chung et al. 2013) R packages, and results summarized using the *Anova* function in the *car* R package (Fox and Weisberg 2019). The *blmer* function allows maximum a posteriori estimation for LME models in a Bayesian setting (Chung et al. 2013). We identified post hoc differences (e.g. D with U1 and U1 with U2) using a least-squares means approach with multiplicity adjustments (Tukey's HSD) obtained from the R package *lsmeans* (Lenth 2016). We calculated standardized mean effect sizes between locations using the *batch_calc_ES* function in the R package *SingleCaseES* (Pustejovsky et al. 2022). We tested contingencies in the magnitude of impacts of WWTP effluents using data including alder litter processing rates (*k*/degree-day) from 60 sampling locations at 20 sites collected over two years (autumn 2013 and 2014). Here we analyzed the relationship between upstream CWM detritivore trait values and site-specific standardized mean effect sizes between locations D–U1 and U1–U2 using linear regression and LME, with the latter employing the *blme* R package described above.

We used variation partitioning analyses to assess the contribution of different influence factors to variation in litter processing rates. We tested the contribution of CWM detritivores ('food' trait modality 'plant detritus > 1 mm') with two *ultimate* anthropogenic pressures (% of treated wastewater in the receiving stream and % arable cropping in the upstream catchment) and four *proximate* stressors (DIN, dissolved inorganic nitrogen, $\mu\text{g l}^{-1}$; SRP, soluble reactive phosphorus, $\mu\text{g l}^{-1}$; TUs of insecticides and fungicides). We used data including alder litter processing rates (*k*/degree-day) from 40 sampling locations at 20 sites collected over two years (autumn 2013 and 2014). The forward-selection procedure recommended by Blanchet et al. (2008) confirmed the importance of CWM detritivores to alder litter processing, with the other predictors selected based on a priori hypotheses (Burdon et al. 2019, 2020). We grouped DIN and SRP together as nutrients for the variation partitioning

analysis. We used the *varpart* (variation partitioning) and *rda* (redundancy analysis) functions in the *vegan* R package (Oksanen et al. 2019) for these analyses. The significance of each independent variation component was permutation-tested using 1000 randomizations (Peres-Neto et al. 2003). Results were visualized with the *venneuler* package in R (Wilkinson 2011).

We used structural equation models (SEM) to test hypotheses about different causal paths leading to impacts on stream litter processing. SEM are probabilistic models that unite multiple predictor and response variables in a single causal network (Lefcheck 2016). We specified SEM to assess the role of two proximate anthropogenic pressures (% of treated wastewater in the receiving stream and % arable cropping in the upstream catchment) and four proximate stressors associated with the pressures (DIN, $\mu\text{g l}^{-1}$; SRP, $\mu\text{g l}^{-1}$; TUs of insecticides and non-insecticide micropollutants) on CWM detritivore trait values and leaf-litter processing rates (*k*/degree-day). We used data including alder litter processing rates (*k*/degree-day) from 40 sampling locations at 20 sites collected over two years (autumn 2013 and 2014). Some constraints were applied a priori to model selection: co-linear variables ($r > 0.75$) were removed where their inclusion would affect the interpretation of the results (e.g. total phosphorus and total nitrogen). Following these initial steps, we used a forward-selection procedure to help select a subset of potential explanatory variables for key endogenous model components (i.e. detritivore trait values and litter processing rates). This selection procedure is performed in two steps to control for the probability of a type I error and overestimation of the explained variance. To prevent overestimation of the explained variance, the forward selection has to be carried out with two stopping criteria: 1) the usual alpha significance level ($\alpha = 0.05$) and 2) the adjusted coefficient of multiple determination (R^2_{adj}) calculated using all potential explanatory variables. Similar stepwise model building approaches have been used to identify variables used in SEM (Harvey and Altermatt 2019, Burdon 2020).

We tested the influence of ammonium in addition to DIN since the unionised form of ammonia (NH_3) is regarded as highly toxic (Russo 1985). However, we found that none of our models selected ammonium as an important variable for further analysis. There were no effects of Metal TUs in our statistical analyses, so we focused on TUs of organic MPs. We found that non-insecticides TUs (i.e. total TUs – insecticide TUs) was a better predictor than fungicide TUs in model iterations. Sediment predictors (total suspendable sediment, % organic sediment) were also considered but excluded during stepwise selection. To achieve SEM parsimony, non-significant paths were removed if the change did not violate Shipley's test of directed separation where we sought $p > 0.05$ to accept the null hypothesis that no important paths were missing in the model. Bayesian information criterion (BIC) scores were compared for all model iterations to check that the final model was the most appropriate. BIC are measures of the relative goodness of fit for a statistical model; the preferred model is the one with the lowest BIC value (Burnham

and Anderson 2002). SEM were fitted with random effects for *Site* nested in *Year* using the R package *piecewiseSEM* (Lefcheck 2016).

In all models, predictor and response variables were transformed to improve normality and homoscedasticity. We logit-transformed proportion data (e.g. % arable cropping), log-transformed water quality predictors and log+1 or square-root transformed invertebrate and litter processing data. Data used for variation partitioning and SEMs was then standardized using the 'deconstand' function in the *vegan* R package (i.e. centered on the column means and scaled by unit variance). All analyses were conducted in R ver. 4.1.2 (<www.r-project.org>). For more detailed descriptions of methods see the Supporting information.

Results

We found striking and highly significant reductions in common alder *Alnus glutinosa* litter processing rates downstream of WWTPs after sampling 20 Swiss streams (Table 1). This change was apparently driven by a decrease in invertebrate-mediated decomposition rates, shown by a significant reduction at downstream wastewater-impacted locations sampled in 2013. In contrast, microbial-mediated decomposition rates (k /days) showed a small, but significant increase at downstream locations. This result was almost entirely explained by the positive influence of warming downstream of the WWTP discharge, as evidenced by the negligible change in temperature-corrected decomposition rates (Table 1). Mean daily stream temperatures were significantly warmer at downstream locations (Table 1), with an average increase over both years of 0.78°C (95% CI, 0.30–1.25°C). The increase in microbial-mediated decomposition did not compensate for the loss in detritivore 'shredding' activity, as demonstrated by the reduced ratio of invertebrates to microbial-mediated decomposition at the downstream locations (Table 1).

Litter processing rates for common oak *Quercus robur* showed similar patterns (Table 2), despite alder leaf bags being closer to the T50 values (50% of mass loss) than the oak leaf bags in 2013 (average of 51% compared to 19% for total mass loss). Litter processing and invertebrate-mediated decomposition rates of oak leaves were significantly reduced at locations below the WWTPs. Microbial-mediated decomposition rates of oak leaves were slower than that of alder (average of 17% compared to 28% mass loss) and did not differ between sampling locations (Table 2). The oak leaf-bags also showed significantly reduced ratios of invertebrate to microbial-mediated decomposition at the downstream locations (Table 2).

We found that the strength of negative wastewater effects on alder litter processing rates (k /degree-day) were contingent on upstream abundances of detritivores (Fig. 1a). Upstream community-weighted mean (CWM) trait values for detritivorous invertebrates drove the size of the negative effect between upstream reference and downstream wastewater-impacted locations ('blmer', $X^2_1 = 6.31$, $p < 0.05$). In contrast, our 'null' model involving the comparison of litter processing rates between the two upstream locations showed no significant associations with upstream detritivores (Fig. 1b).

Variation partitioning indicated the contribution of detritivorous invertebrates to alder litter processing rates (Fig. 2). We found that the proportion of wastewater in the receiving stream contributed to 4.02% of the variation in litter processing rates (Fig. 2a), although the independent influence of % wastewater was not significant. The proportion of arable cropping in the upstream catchment was not directly important for alder litter processing rates. Nutrients (SRP and DIN), insecticides and fungicides all explained small fractions of variation in litter processing that was shared with detritivorous invertebrates (Fig. 2b). There were no significant independent influences of nutrients, insecticides and fungicides on alder litter processing rates.

Table 1. Results of the leaf-bag assay measuring leaf-litter processing rates of alder *Alnus glutinosa* leaves at sampling locations downstream (D) and upstream (U1, U2) of wastewater discharges in 20 Swiss streams sampled during autumn 2013 ($n = 12$) and 2014 ($n = 8$). Mean values are presented ± 1 SD. Mean daily stream temperatures (°C) are presented. The k -ratio is a dimensionless metric calculated using the $\log(1+x)$ -transformed ratio of coefficients from invertebrate and microbial mediated decomposition (Eq. 4). Standardised mean differences (SMD ± 1 SE) quantify differences in responses downstream (D) compared to upstream (U1). F-statistics, degrees of freedom (df), p-values and the proportion of variance explained by the random effect are presented from mixed-model ANOVAs where the sampling location (U1, U2, D) was a fixed factor. R^2_r indicates the variance explained by the random effects. Litter processing rate coefficients k day⁻¹ are presented $\times 10^2$; k degree-day⁻¹ $\times 10^3$.

Year(s)	Response	Unit	Sampling location			SMD	F-stat	df	p-value	R^2_r
			U2	U1	D					
All	k_{total}	day ⁻¹	2.49 \pm 1.78	2.62 \pm 1.61	2.00 \pm 0.89	-0.67 \pm 0.46	6.45	2,322	0.002	63
		degree-day ⁻¹	3.40 \pm 2.31	3.52 \pm 1.97	2.52 \pm 1.09	-0.88 \pm 0.46	14.8	2,322	< 0.001	61
2013	k_{total}	day ⁻¹	2.74 \pm 2.11	2.47 \pm 1.75	1.98 \pm 1.06	-0.43 \pm 0.52	5.94	2,188	0.003	63
	$k_{\text{invertebrate}}$		1.96 \pm 2.06	1.66 \pm 1.63	1.18 \pm 1.09	-0.41 \pm 0.49	7.74	2,182	< 0.001	66
	$k_{\text{microbial}}$		0.84 \pm 0.18	0.83 \pm 0.14	0.91 \pm 0.20	0.38 \pm 0.34	4.70	2,186	0.010	56
	k_{total}	degree-day ⁻¹	4.13 \pm 2.58	3.77 \pm 2.13	2.87 \pm 1.17	-0.72 \pm 0.58	9.20	2,188	< 0.001	49
	$k_{\text{invertebrate}}$		2.87 \pm 2.65	2.45 \pm 2.11	1.65 \pm 1.35	-0.55 \pm 0.51	9.39	2,182	< 0.001	59
	$k_{\text{microbial}}$		1.36 \pm 0.20	1.35 \pm 0.13	1.36 \pm 0.14	0.00 \pm 0.36	0.02	2,186	0.984	26
	k -ratio	dimensionless	0.94 \pm 0.58	0.86 \pm 0.55	0.67 \pm 0.42	-0.43 \pm 0.45	9.42	2,178	< 0.001	59
All	Stream temperature	°C	7.43 \pm 1.82	7.38 \pm 1.82	8.19 \pm 2.39	0.32 \pm 0.27	19.8	2,38	< 0.001	36
2013	Stream temperature	°C	6.24 \pm 1.18	6.16 \pm 1.08	6.66 \pm 1.24	0.38 \pm 0.36	17.2	2,22	< 0.001	95

Table 2. Results of the leaf-bag assay measuring leaf-litter processing rates of common oak *Quercus robur* leaves at sampling locations downstream (D) and upstream (U1, U2) of wastewater discharges in 12 Swiss streams sampled during autumn 2013. Mean values are presented ± 1 SD. Mean daily stream temperatures ($^{\circ}\text{C}$) are presented. The k -ratio is a dimensionless metric calculated using the $\log(1+x)$ -transformed ratio of coefficients from invertebrate and microbial mediated decomposition (Eq. 4). Standardised mean differences (SMD ± 1 SE) quantify differences in responses downstream (D) compared to upstream (U1). F-statistics, degrees of freedom (df), p-values and the proportion of variance explained by the random factor (Stream) are presented from mixed-model ANOVAs where the sampling location (U1, U2, D) was a fixed factor. R^2 indicates the variance explained by the random effect (Stream). Litter processing rate coefficients $k \text{ day}^{-1}$ are presented $\times 10^2$; $k \text{ degree-day}^{-1} \times 10^3$.

Response	Unit	Sampling location			SMD	F-stat	df	p-value	R^2
		U2	U1	D					
k_{total}	day^{-1}	0.58 ± 0.12	0.58 ± 0.16	0.54 ± 0.18	-0.22 ± 0.36	3.73	2,192	0.026	58
$k_{\text{invertebrate}}$		0.12 ± 0.07	0.13 ± 0.09	0.10 ± 0.12	-0.23 ± 0.34	4.13	2,183	0.018	41
$k_{\text{microbial}}$		0.48 ± 0.09	0.47 ± 0.09	0.48 ± 0.09	0.12 ± 0.40	0.25	2,181	0.777	49
k_{total}	degree-day^{-1}	0.93 ± 0.08	0.95 ± 0.16	0.81 ± 0.17	-0.79 ± 0.40	13.2	2,192	< 0.001	23
$k_{\text{invertebrate}}$		0.19 ± 0.09	0.21 ± 0.12	0.14 ± 0.15	-0.42 ± 0.36	5.77	2,183	0.004	34
$k_{\text{microbial}}$		0.78 ± 0.12	0.77 ± 0.07	0.74 ± 0.11	-0.30 ± 0.33	2.53	2,181	0.083	27
k -ratio	dimensionless	0.22 ± 0.12	0.23 ± 0.11	0.17 ± 0.16	-0.35 ± 0.34	3.17	2,179	0.044	26

We used structural equation modelling (SEM) to test causal influences of different anthropogenic drivers on detritivorous invertebrates and their contribution to ecosystem functioning, as measured by litter processing rates ($k/\text{degree-day}$) of alder leaf litter. Detritivore abundances, indicated by mean trait values, had a positive effect on litter processing rates (Fig. 3). In turn, detritivores were negatively influenced by toxic units of insecticides and positively associated with dissolved phosphorus

concentrations. The wastewater fraction in the receiving stream had a direct negative influence on litter processing (Fig. 3). Wastewater also had positive effects on nutrients and micropollutants, whereas increasing proportions of upstream land covered for arable cropping were positively associated with insecticides and nitrogen concentrations. In the SEM, we were able to account for upstream land use impacts, thus helping to explain contingencies in wastewater impacts observed in Fig. 1a.

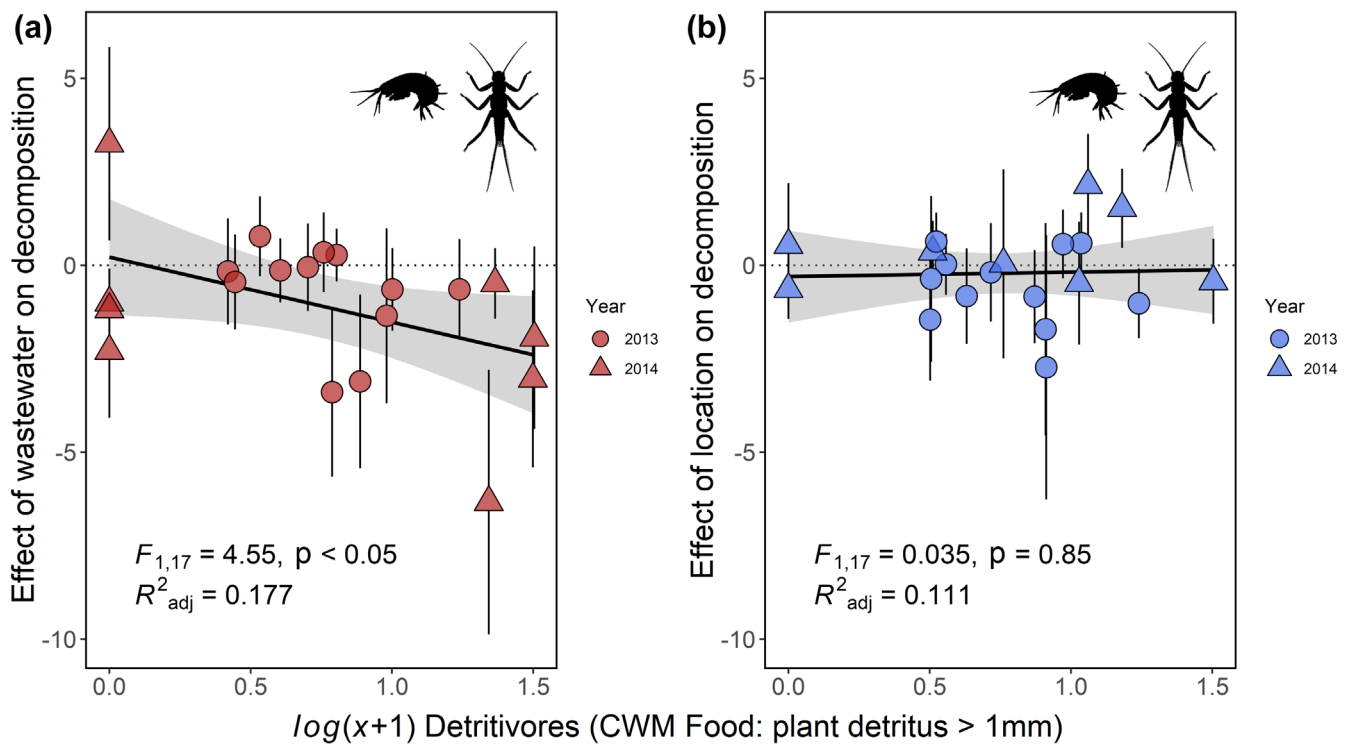


Figure 1. Effects of wastewater on leaf-litter processing are context dependent. (a) Across 20 Swiss streams, the strength of negative wastewater impacts on litter breakdown rates were contingent on abundances of detritivores at the upstream reference location. (b) In contrast, there was no change between the two upstream sampling locations. Effect sizes (standardised mean differences) include the 95% confidence interval and contrast alder litter breakdown rates ($k/\text{degree-days}$) between sampling locations (a: U1–D; and b: U2–U1). Invertebrate abundances from leaf bags at U1 (a) and U2 (b) were used to calculate community-weighted mean (CWM) values for the ‘food’ trait modality ‘plant detritus > 1 mm’ (Usseglio-Polatera et al. 2000). Results from individual linear regression models are presented in each panel, but we also tested patterns with a Bayesian linear mixed-effects model.

Partial regression plots (Fig. 4) helped to show the data underpinning the relationships elucidated in the SEM. There was a significant positive influence of SRP on detritivorous invertebrates after accounting for the negative influence of insecticides (Fig. 4a). In contrast, insecticides had a significant negative influence on detritivorous invertebrates after accounting for the influence SRP (Fig. 4b). The proportion of wastewater in the receiving stream had a significant negative influence on litter processing after accounting for detritivorous invertebrates (Fig. 4c). Conversely, detritivorous invertebrates has a significant positive influence on litter processing after accounting for the negative influence of treated wastewater (Fig. 4d).

Several environmental predictors were colinear (Fig. C1). Notably, DIN concentrations were highly correlated with nitrate ($r=0.998$, $t_{38}=94.8$, $p < 0.001$), and non-insecticide TUs were correlated with fungicide TUs ($r=0.644$, $t_{38}=5.19$, $p < 0.001$). For more results describing environmental drivers and litter processing responses see the Supporting information.

Discussion

We found evidence for widespread negative impacts of wastewater inputs on stream ecosystem functioning as measured by leaf-litter processing rates. Negative influences on invertebrate-mediated decomposition drove this adverse ecosystem response, and the strength of the impact tended to be contingent on upstream abundances of invertebrate consumers. In contrast, wastewater effects on microbial-mediated decomposition were mostly neutral after accounting for the warming influence of the effluent input. This meant litter processing rates were inextricably bound to the abundances of detritivorous invertebrates, which in turn appeared to be susceptible to negative influences of insecticides emanating from the wastewater treatment plants and arable cropping in the upstream catchment. Our study indicates how the pervasive toxic threat

from synthetic chemical pollution can ripple through ecosystems, with asymmetric impacts on key food-web compartments culminating in altered system functioning.

In our previous study, we showed that nutrient concentrations were greatly increased below WWTPs (Burdon et al. 2019). In the present study, we saw neutral or negative effects of effluent inputs on litter processing after accounting for the warming effect of these discharges – suggesting that nutrient enrichment did not have a strong positive influence on litter decomposition rates (Table 1). A meta-analysis has shown that nutrient enrichment can greatly stimulate litter decomposition, particularly in nutrient-poor streams, increasing rates by an average of ~50% (Ferreira et al. 2015). None of our streams had dissolved nitrogen concentrations low enough to expect a stimulation of decomposition by nitrogen enrichment, and only five streams had phosphorus concentrations marginally below the range found sufficient to satisfy microbial nutrient demands (Ferreira et al. 2015). In our study, we used litter from a nitrogen-fixing tree (*A. glutinosa*) that provides a large nitrogen pool to microbial decomposers, potentially freeing them from limitation of this element in the environment (Boyer et al. 2011). The relatively high background concentrations of nutrients, combined with alder leaf stoichiometry help explain weak effects of nutrients in treated effluent on microbial-mediated alder litter processing rates. In contrast, oak (*Q. robur*) decomposed at a slower rate than alder. Alder and oak have contrasting physical and chemical characteristics (greater softness, greater concentrations of nutrients and lower concentrations of structural and secondary compounds in alder than in oak) and thus differ greatly in their palatability to litter-consuming detritivores (Chauvet et al. 2016). These differences help to explain why our litter processing assay for this tree species was less sensitive to wastewater inputs.

However, potential antagonistic interactions with other wastewater-born toxicants (e.g. fungicides) should not be

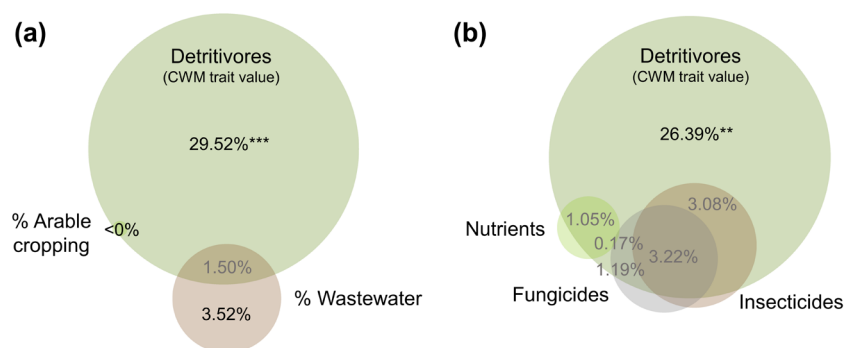


Figure 2. Variation-partitioning analysis of factors contributing to alder leaf-litter breakdown ($k/\text{degree-days}$) at two sampling locations (D, U1) in 20 Swiss streams sampled autumn 2013 ($n=12$) and 2014 ($n=8$). (a) Invertebrate detritivores: community-weighted mean values for the 'food' trait modality 'plant detritus > 1 mm' (Usseglio-Polatera et al. 2000) calculated from leaf-bag invertebrates, and two ultimate environmental drivers: % wastewater, proportion of treated wastewater in the receiving stream; % arable cropping, proportion of arable cropping in the upstream catchment. (b) Invertebrate detritivores and three proximate environmental drivers that included nutrients: DIN, dissolved inorganic nitrogen ($\mu\text{g l}^{-1}$) and SRP, soluble reactive phosphorus ($\mu\text{g l}^{-1}$); fungicides, toxic units; and insecticides, toxic units. Variation < 0% is not shown unless specified. The unexplained (residual) variation of model (a) = 68.08%; model (b) = 74.38%. *** $p < 0.001$, ** $p < 0.01$.

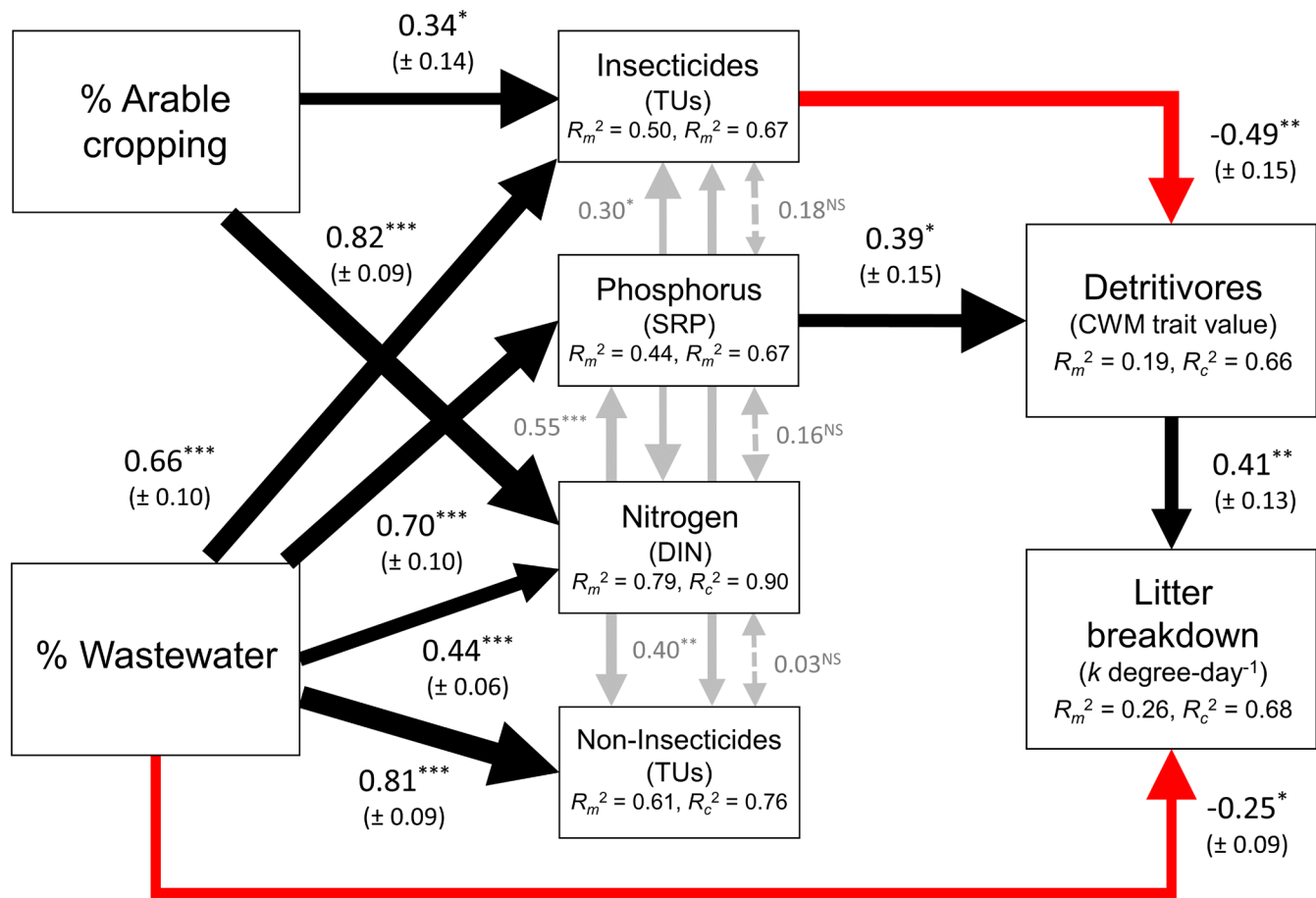


Figure 3. Negative effects of insecticides on detritivore communities' cascade to ecosystem-level impacts on leaf-litter processing. Insecticides associated with inputs of treated wastewater and arable cropping negatively influence the abundances of detritivores, which in turn positively influence litter breakdown. This structural equation model (SEM) uses alder litter breakdown (k /degree-days), community-weighted mean (CWM) values for the 'food' trait modality 'plant detritus > 1 mm' (Usseglio-Polatera et al. 2000) calculated from leaf bag invertebrates, and environmental data from two sampling locations (D,U1) at 20 Swiss streams sampled in autumn 2013 and 2014. DIN, dissolved inorganic nitrogen ($\mu\text{g l}^{-1}$); SRP, soluble reactive phosphorus ($\mu\text{g l}^{-1}$); TU, toxic units; % wastewater, proportion of treated wastewater in the receiving stream; % arable cropping, proportion of arable cropping in the upstream catchment. Bolded lines indicate significant positive (black) and negative (red) relationships. Grey dashed lines indicate non-significant relationships, grey double-headed lines indicate correlated error terms. The width of the lines is standardised and thus indicates the relative strength of the relationships with standardised path coefficients indicated (± 1 SE). Marginal R^2 values (R_m^2) indicate the goodness of fit for endogenous variables excluding variance explained by the random effects, Conditional R^2 values (R_e^2) indicate variance explained by the fixed and random effects. Model statistics: Fisher's $C = 9.67$, $p = 0.989$, $df = 22$, $\Delta\text{BIC} = 0.994$. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

discounted (Burdon et al. 2020). In particular, the stimulatory effect of nutrients on microbial-mediated decomposition could be cancelled by an inhibitory effect from another wastewater constituent. For example, Feckler et al. (2018) found that the effects of nutrients in the presence of increased concentrations of fungicides depended on microbial community history: pollution-induced community tolerance in a previously disturbed microbial community, which was dominated by a few hyphomycete species with high litter processing efficiencies, potentially explained the maintenance of decomposition under stress. We did not see similar positive functional resilience in our study, which may reflect stress-induced community sensitivity where negative species co-tolerances reduced or reversed positive responses to additional stressors (Vinebrooke et al. 2004).

Our results support previous research showing that pesticides are important contributors to the toxic pressure in these wastewater-impacted streams (Munz et al. 2017). At the same sites where the present study was conducted, the organophosphate insecticide diazinon was frequently detected (> 20%) downstream of the WWTPs with concentrations peaking at 130 ng l^{-1} . This peak concentration was determined off a relatively modest number of grab samples, and given the dynamic and episodic nature of pesticide concentrations in receiving environments it could be a conservative estimate of actual maximum values (Munz et al. 2017). When considering the acute threat posed by concentrations (ng l^{-1}) of MPs to stream invertebrate detritivores, we found that diazinon and the neonicotinoid insecticide clothianidin disproportionality contributed to the toxic risk. These results were supported by

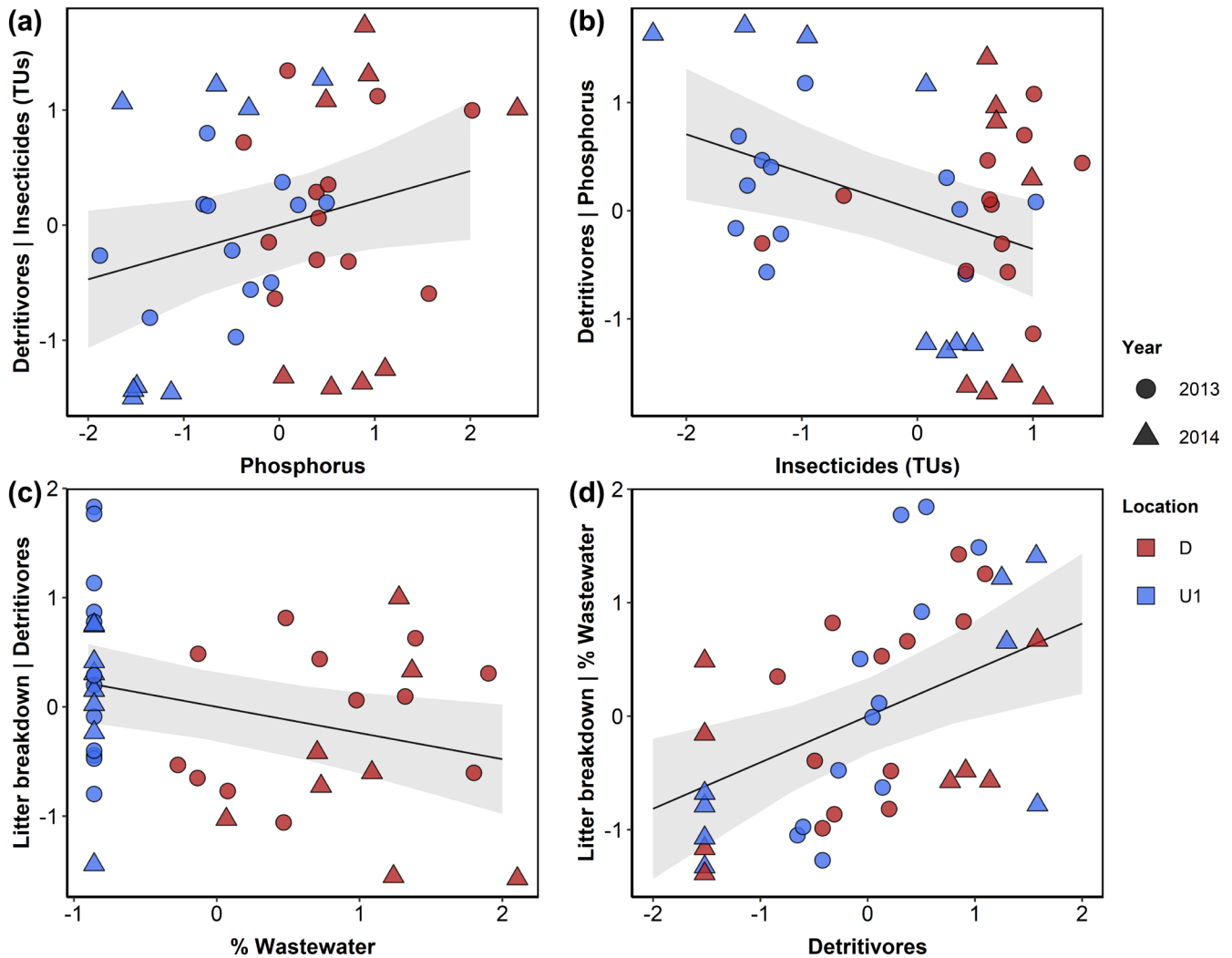


Figure 4. Partial regression plots from key relationships elucidated in the SEM (Fig. 3). (a) The influence of phosphorus (SRP, $\mu\text{g l}^{-1}$) on community-weighted mean values for the 'Food' trait modality 'plant detritus > 1 mm' (detritivores) after accounting for the influence of insecticide TUs (toxic units). (b) The influence of insecticide TUs on detritivores after accounting for the influence of phosphorus (DRP). (c) The influence of the proportion of treated wastewater in the receiving stream (% wastewater) on alder litter breakdown ($k/\text{degree-days}$) after accounting for the influence of detritivores. (d) The influence of detritivores on litter breakdown after accounting for the influence of % wastewater. Regression lines and 95% confidence limits are from linear-mixed models; parameter estimates are provided in the Supporting information.

bioassays of acetylcholinesterase inhibition at the same study sites (Kienle et al. 2019). Insecticides generally target the nervous system of macroinvertebrate detritivores (e.g. gammarid amphipods) mediated by acetylcholine neurotransmitters, making bioassays that measure the functioning of the enzyme acetylcholinesterase useful diagnostic tools for monitoring the putative threat of these chemicals to populations and ecosystems (Lionetto et al. 2013). In Kienle et al. (2019), mean parathion equivalent concentrations (indicating the degree of AChE inhibition) increased from 249 ng l^{-1} upstream to 411 ng l^{-1} downstream, indicating that the corresponding effects-based trigger (EBT) of 196 ng l^{-1} could be exceeded, particularly in the presence of WWTP inputs.

Detrital consumption by individual detritivores (e.g. gammarid amphipods) may be impaired in the presence of

wastewater due to negative influences of micropollutants (Bundschuh et al. 2011), and Münze et al. (2017) correlated reductions of pesticide-sensitive stream invertebrates with slower rates of litter processing. At the same sites where the present study was conducted, micropollutants including neonicotinoids have frequently been detected internally in gammarid amphipods (Munz et al. 2018). The sum internal MP concentrations of gammarids ranged from < LOQ (limit of quantification) to 16 ng g^{-1} wet weight (w.w.) at upstream sites and < LOQ to 86 ng g^{-1} w.w. at downstream sites. The neonicotinoid thiacloprid was frequently detected (> 20 detections) with a highest maximum concentration of 21 ng g^{-1} w.w., and a median concentration of 4.9 ng g^{-1} w.w., but other neonicotinoids (e.g. imidacloprid) were also disproportionately present in gammarids downstream of the WWTPs

(Munz et al. 2018). Gammarid internal MP concentrations were higher in autumn than in spring, indicating that toxic pressure could have been greater when we conducted our experiment. Münze et al. (2017) similarly showed pesticide toxicity due to WWTP inputs increasing over summer, with the neonicotinoids imidacloprid and thiacloprid featuring prominently as potential drivers of ecological harm.

Variation in micropollutant prevalence through time could have implications for ecosystem functioning, if these toxicants asymmetrically affect different life-stages of stream detritivores. In Taddei et al. (2021), we used an laboratory experiment to highlight the size-dependent sensitivity of gammarid amphipods when exposed to realistic mixtures of micropollutants. The negative effects on juvenile gammarids could help explain impaired stream ecosystem functioning due to their greater relative abundances and metabolic demands during autumn. However, our present study is the first to demonstrate the ecosystem consequences of these impacts across a broad range of sites using a mechanistic framework, whilst showing how the magnitude of WW-impacts are contingent on detritivore populations and upstream environmental quality. We found evidence of direct negative effects of wastewater on litter processing rates (Fig. 3), which could be mediated through 'cryptic function loss' (McConkey and O'Farrill 2015) owing to the non-lethal effects of toxicants on detritivore performance (Bundschuh et al. 2011, Taddei et al. 2021). Direct negative effects of WW could also be due to unmeasured stressors. Despite the broad range of compounds considered in our study, important groups such as pyrethroid pesticides were excluded from characterisation in the micropollutant load (Burdon et al. 2019). These chemicals are highly toxic to invertebrates, but were not measured because they are not amenable to the analysis method (LC-HRMS) used (Munz et al. 2017).

We did not find significant negative effects of metals, despite their elevated concentrations in these streams downstream of the WWTPs (Munz et al. 2017, Burdon et al. 2019). More intensive sampling could help resolve this question, given that other studies have pointed to toxic effects of metals in treated effluent (Beasley and Kneale 2002, Stalter et al. 2013). Other unmeasured stressors potentially contributing to negative WW effects include effluent-borne microorganisms (Mansfeldt et al. 2020, Carles et al. 2021), which may directly (e.g. pathogenic, microbiome dysbiosis) and indirectly (e.g. oxygen depletion in leaf bags) alter consumer–resource interactions (Burdon et al. 2020, Millar et al. 2021). We observed blackening of leaves in fine-mesh leaf bags downstream of the WWTPs with some also showing signs of an orange precipitate forming at the leaf margins. Our observations point to microbial activity and in particular, bacteria associated with different oxic states. Oxygen limitation may create anoxic conditions, which allows anaerobic bacteria to decompose organic matter (Nealson 1997). Ferrous iron in a reduced form can be responsible for the blackening of organic matter due to the activity of anaerobic microorganisms (Burdige 2006). Further, the accumulation of orange ferric iron in freshwaters can be due to bacteria such as *Leptothrix* and *Sphaerotilus* (Johnson and Hallberg 2005). However, despite

these putative alternative stress pathways, we found evidence for negative impacts of chemical pollution and linked this ecological harm to the class of compounds (i.e. insecticides) that pose the greatest specific threat to invertebrates.

We have presented evidence for how widespread human pressures (i.e. treated wastewater and arable cropping) exert negative effects on stream community structure and ecosystem functioning. Our study helps to explain contingencies in the strength of negative wastewater impacts by demonstrating how diffuse chemical pollution (e.g. insecticides) from the surrounding landscape shapes the communities exposed to point-source pollution. The pervasive impacts of insecticides from multiple sources further indicate the threat these bioactive chemicals pose in surface waters (Stehle and Schulz 2015). Our results indicate how biodiversity loss (i.e. functional traits) driven by exposure to insecticides can exceed functional redundancies across trophic levels in the environment. Boyero et al. (2021a) showed a positive relationship between diversity and decomposition. Their results suggest that the loss of detritivore biodiversity may alter litter processing at global scales, with effects strongest in tropical areas characterised by low detritivore diversity and a prevalence of environmental stressors including pollution. With land use intensity and demands on natural resources set to increase in the face of ongoing global change (Tilman et al. 2017), it is critically important to simultaneously manage surface waters sustainably and meet the demands of biodiversity conservation and environmental legislation (Woodward et al. 2012, Harrison et al. 2018).

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Author contributions

Francis J. Burdon: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (lead); Methodology (equal); Project administration (equal); Validation (equal); Visualization (equal); Writing – original draft (lead); Writing – review and editing (equal). **Marta Reyes:** Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Urs Schönenberger:** Data curation (supporting); Formal analysis (supporting); Validation (supporting). **Katja Räsänen:** Conceptualization (equal);

Methodology (equal); Project administration (supporting); Supervision (equal); Writing – review and editing (equal). **Scott D. Tiegs:** Investigation (supporting); Methodology (equal); Writing – review and editing (equal). **Rik I. L. Eggen:** Conceptualization (equal); Funding acquisition (equal); Supervision (equal); Writing – review and editing (equal). **Christian Stamm:** Conceptualization (equal); Formal analysis (supporting); Funding acquisition (equal); Methodology (equal); Project administration (equal); Supervision (equal); Validation (equal); Writing – review and editing (equal).

Data availability statement

Data and code will be made available via the Dryad Digital Repository <<https://doi.org/10.5061/dryad.ncjsxksv8>> (Burdon et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

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