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Agriculture versus wastewater pollution as drivers of macroinvertebrate community structure in streams

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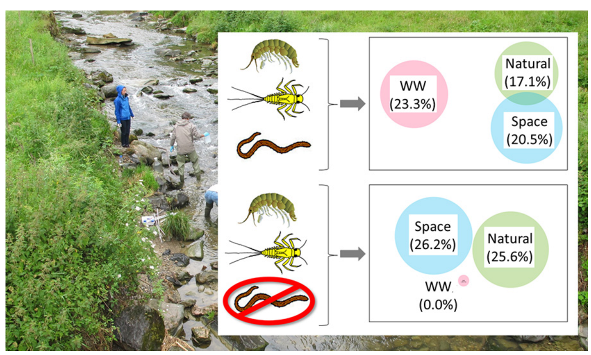
HIGHLIGHTS

- We studied wastewater impact on 23 headwater streams across a broad land use gradient.
- Water quality and modified habitat explained 30% and 13% of the community composition.
- Impacts of organic micropollutants on macroinvertebrates were clearly detectable.
- Except for oligochaetes agricultural pollution had a stronger impact than wastewater.
- Wastewater had little influence except on oligochaetes suggesting efficient treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Water pollution is ubiquitous globally, yet how the effects of pollutants propagate through natural ecosystems remains poorly understood. This is because the interactive effects of multiple stressors are generally hard to predict. Agriculture and municipal wastewater treatment plants (WWTPs) are often major sources of contaminants for streams, but their relative importance and the role of different pollutants (e.g. nutrients or pesticides) are largely unknown. Using a 'real world experiment' with sampling locations up- and downstream of WWTPs, we studied how effluent discharges affected water quality and macroinvertebrate communities in 23 Swiss streams across a broad land-use gradient.

Variation partitioning of community composition revealed that overall water quality explained approximately 30% of community variability, whereby nutrients and pesticides each independently explained 10% and 2%, respectively. Excluding oligochaetes (which were highly abundant downstream of the WWTPs) from the analyses, resulted in a relatively stronger influence (3%) of pesticides on the macroinvertebrate community composition, whereas nutrients had no influence. Generally, the macroinvertebrate community composition downstream of the WWTPs strongly reflected the upstream conditions, likely due to a combination of efficient treatment processes, environmental filtering and organismal dispersal. Wastewater impacts were most prominently by the Saprobic index, whereas the SPEAR index (a trait-based macroinvertebrate metrics reflecting sensitivity to pesticides) revealed a strong impact of arable cropping but only a weak impact of wastewater.

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Overall, our results indicate that agriculture can have a stronger impact on headwater stream macroinvertebrate communities than discharges from WWTP. Yet, effects of wastewater-born micropollutants were clearly quantifiable among all other influence factors. Improving our ability to further quantify the impacts of micropollutants requires highly-resolved water quality and taxonomic data with adequate spatial and temporal sampling. These improvements would help to better account for the underlying causal pathways that drive observed biological responses, such as episodic contaminant peaks and dispersal-related processes.

1. Introduction

Global biodiversity is changing at an unprecedented rate in response to human activities (Sala et al., 2000; World Resources Institute, 2005). Aquatic ecosystems are particularly affected by human pressures including habitat degradation, water abstraction, and pollution from industrial, agricultural, and urban sources (Allan, 2004; Friberg, 2010; Vörösmarty et al., 2010). Impaired water quality due to nutrients, fine inorganic sediment, and/or micropollutants (MPs[1]) have been reported to correlate with negative ecological effects (Liess and von der Ohe, 2005; Smith et al., 2006; Burdon et al., 2013; Sundermann et al., 2015). However, a major challenge in the protection and management of aquatic ecosystems is understanding the *relative* contribution of different anthropogenic pressures so that effective mitigation strategies can be developed. Although developed countries have greatly reduced nutrient impacts from point-sources of water pollution (e.g., wastewater discharges), convential treatment processes are not designed to deal with the growing number of chemicals present in the untreated effluent (Vaughan and Ormerod, 2012; Stamm et al., 2016). Furthermore, diffuse sources of contaminants from human land-uses often remain persistent threats, thus confounding our ability to quantify point-source impacts and the underlying stressor pathways (Burdon et al., 2016).

Managing multiple stressors can be difficult because they often act simultaneously and interactively (Townsend et al., 2008). Stressor interaction effects may not only be additive, but 'ecological surprises' may arise through synergistic or antagonistic outcomes (Jackson et al., 2016; Brittain and Strecker, 2018). In the context of water pollution, additional stressors can increase the sensitivity of organisms to toxicants (Relyea, 2003; Liess et al., 2016). Thus, biotic responses to human activities (e.g. chemical pollution) may be strongly contingent on environmental factors, such as the magnitude of co-occurring stressors and the distribution of strong and weak interactions in ecological networks (Burdon et al., 2016; Wootton and Stouffer, 2016).

The prevalence of multiple stressors raises the question of how best to disentangle the causal and relative role of different factors, so as to provide a sound basis for deciding how to optimally allocate resources for mitigation. Ideally, the role of stressors would be studied in large-scale experiments to establish causalities, but the logistical challenges and lack of resources typically result in most studies either being comparative field studies (i.e., large-scale) or highly constrained experiments (i.e., small-scale). An alternative approach are 'real world experiments'. These make use of altered ecological conditions in natural settings, thus allowing powerful inferences by combining the benefits of ecologically realistic conditions, manipulations and replication (Diamond, 1983; Stamm et al., 2016).

To disentangle the role of wastewater (WW) discharge in affecting macroinvertebrate communities in the context of other influence factors, we compared differences between two upstream (U1, U2) and one downstream (D) location in 24 Swiss streams impacted by conventionally-treated wastewater. Macroinvertebrates were chosen as a target group as they are sensitive indicators of environmental conditions and used in numerous river health assessment programs (see e.g., Stucki, 2010; Kunz et al., 2016). Our study further developed the analysis introduced in Burdon et al. (2016). A major strength compared to this earlier study is that we also quantified organic micropollutants (MPs, e.g. pesticides and pharmaceuticals) as well as heavy metals (HMs) and increased the level of replication from 12 to 24 sites. This provides a more rigorous basis to statistically tease apart which wastewater constituents likely exert the observed biological effects. It also allows for quantifying the role of chemicals stressors in the context of other influencing factors by means of variance partitioning models (VP). We predicted that macroinvertebrate responses to WW would be greatest in the streams where upstream human impacts were the lowest (Burdon et al., 2016). We also expected that quantifying the concentrations and toxicity of MPs and HMs would allow insight to underlying stressor pathways (e.g., toxic effects versus consequences of different nutrient levels) contributing to observed WW effects.

2. Methods

2.1. Site selection and study design

We selected 24 streams across Switzerland (Fig. 1, one site was finally discarded, see below) that each received effluent from a single wastewater treatment plant (WWTP) using the same criteria described in Burdon et al. (2016) and Stamm et al. (2016). Briefly, sites were selected so that no other WWTPs were located upstream and there was a minimum flow contribution of 20% wastewater downstream of the WWTPs (i.e., at sampling location D, see below) during low flow conditions (Q_{347}, see below). The selected WWTPs varied in treatment technology, resulting in different levels of nutrient removal and subsequent pollution to receiving streams (see Fig. S10). The study streams were small to medium sized, with catchment areas[2] ranging from 6.8 to 65.9 km^2. Catchments were distributed across three Swiss biogeographical regions (Swiss Plateau, Jura, and Pre-alps) and differed considerably in land use composition (Table S1).

At each study site, we chose one downstream sampling location (D) as an impacted site, and two upstream sampling locations (U1, U2) as reference sites unimpacted by WW (Burdon et al., 2016). At each site, the three sampling locations shared a similar river morphology. Importantly, we used the two upstream reference sites to help quantify natural variation between sampling locations (i.e., the change between two sites without an additional pressure, akin to a 'null' model). This approach strengthens the power of our inference when considering the downstream influence of WW at the impacted location (D). Further details on the experimental layout can be found in in Burdon et al. (2016), Stamm et al. (2016), and Munz et al. (2017).

[1] Abbreviations: D: location downstream, DOC: dissolved organic carbon, EC: Effect concentrations, HM: Heavy metals, MPs: organic micropollutants, Q_{347}: discharge statistically exceeding on 347 days per year, Q_{WW}: annual average discharge from a given WWTP, SEM: structural equation modelling, SI: supporting information, SRP: soluble reactive phosphorous, TSS: total suspendible sediments, TU: toxic units, U1: location upstream 1, U2: location upstream 2, VP: variation partitioning, WW: wastewater, WWTP: wastewater treatment plant.

[2] Here we consider the upstream hydrologic catchment areas in contrast to Burdon et al. (2016), where the catchment area of the WWTPs was considered. This difference also causes the land use composition to slightly deviate from the previous paper.

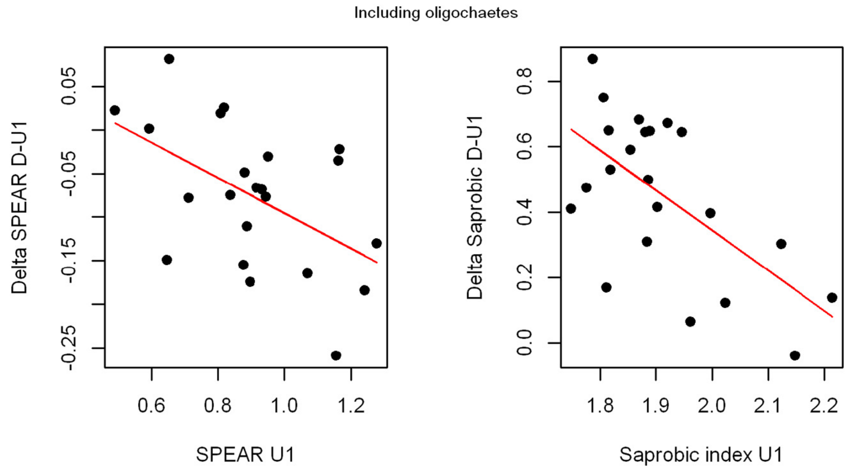


Fig. 1. Relationship between the upstream SPEAR index (left) and Saprobic index (right) and the change to the respective downstream locations. The red lines represent the Pearson correlations (SPEAR index: adjusted $R^2 = 0.227$, p-value = 0.014, saprobic index: adjusted $R^2 = 0.352$, p-value = 0.002).

2.2. Sampling and analysis of physicochemical parameters

A subset of 12 sites were studied in 2013, the other 12 sites in 2014. This separation was done for logistic reasons and the choice of sites/year was random. For the 2013 sites, we took monthly grab samples at each sampling location during low flow conditions. All samples were analyzed for 20 general water quality parameters (Table S2). For the first group of 12 sites (i.e., the '2013' sites), monthly sampling started in March 2013 for downstream (D) and upstream sites (U1), and in April 2013 for the most upstream (U2) locations, and continued until February 2014. Samples for the analysis of organic MPs (two campaigns in June 2013 and January 2014 screening for 389 compounds, see Table S2.1 in (Munz et al., 2017)) were collected on two sampling dates (June 2013 and February 2014) at U1 and D (see Munz et al. (2017) for further details).

Based on the results from 2013, the sampling scheme for the second group of 12 sites (i.e., the '2014 sites') was slightly modified by changing sampling frequency to bi-monthly. For the 2014 sites, all samples were analyzed for the 20 general water quality parameters, MPs and - additionally - heavy metals (HMs). For MP analyses of 2014, a standard compound set was determined (based on 2013 results) consisting of 28 plant protection products, 3 biocides, 22 pharmaceuticals and personal care products, 3 additional household chemicals (see Table S2.2 in Munz et al. (2017))

All water samples (except for HM analyses) were collected in 1 L glass bottles, and transported on ice before storage in the laboratory at Eawag, Dübendorf. Samples for general water chemistry were kept at 4 °C until analyses were performed (within 24 h of collection). These analyses used standard methods described for the Swiss National River Monitoring and Survey Programme (NADUF; www.bafu.admin.ch/wasser/13462/14737/15108/15109). Samples for analyzing organic MPs were frozen at −20 °C and measured with LC-high resolution mass-spectrometry according to the procedure described in (Munz et al., 2017). For HM analyses, samples were filtered (0.45μm) and acidified (0.65% HNO_3) on site and stored in 20 mL Falcon tubes at 4 °C until analysis. HMs were quantified with high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS; Element2, Thermo, Switzerland). See Meylan et al. (2003) for additional details of this method.

Wastewater quantity was calculated as the proportion of treated effluent in the receiving stream below the point of discharge:

$$\%WW = \frac{Q_{ww}}{Q_{347}} \times 100 \tag{1}$$

where Q_{347} is the stream discharge that is reached or exceeded 347 days per year averaged over 10 years (equivalent to 95% of the time) and Q_{WW} is the mean discharge from the WWTP (Table S1).

At each sampling location, water depth and flow velocity were recorded (FlowTracker Handheld ADV, Sontek/YSI, Inc., San Diego, CA, USA) at 10 equidistant points across three transects (also measuring wetted channel width). Total benthic suspendible sediment ($SS_{benthic}$; kg/m^2) and its organic and inorganic fractions (Clapcott et al., 2011; Burdon et al., 2013) were also recorded. For more details on the methods used for $SS_{benthic}$ see SI M1. All these measurements were conducted during low flow conditions in autumn 2013/2014. Additional local habitat characteristics of each stream reach were recorded at the time of macroinvertebrate sampling (see below) according to Swiss biomonitoring protocols (Stucki, 2010). These included benthic substrate composition (inorganic and organic categories) and a discrete ranking of human alterations to instream and riparian properties (Stucki, 2010).

2.3. Measuring and estimating the effects of MPs

The toxic stress at each sampling location was evaluated using the Toxic Unit (TU) approach (Sprague, 1971) which assumes toxic effects are additive. The effect concentration (EC_{50}) for each compound was derived as the geometric mean of all available data on acute toxicity values for aquatic invertebrates (De Zwart, 2002; Munz et al., 2017).

The TUs for each substance *i* (MPs and metals) at each location and time point were calculated by dividing the measured concentrations C_{obs} by the relevant EC50 value of the given substance:

$$TU_i = \frac{C_{obs\_i}}{EC_{50\_i}} \tag{2}$$

Mixture toxicity was calculated by summing up all TUs at each location and time point:

$$sumTU = \sum_i TU_i \tag{3}$$

In total, TUs could be calculated for 129 substances including important pesticides, pharmaceuticals, household chemicals and HMs (see Munz et al. (2017) for further details). However, gaps in this data arise as potentially important toxicants (such as pyrethroid insecticides) were not measured or no EC_{50} value were available for some substances (especially for pharmaceuticals).

For the further analyses, the maximum sumTU (over all time points at one location) for three groups of chemicals (HMs, pesticides, other organic MPs) were used for each location. (Note that using the median sumTU values of all time points per location instead resulted in very similar findings, see Fig. S7–S9).

2.4. Benthic invertebrates

The invertebrate sampling methods followed standard protocols for benthic macroinvertebrate biomonitoring in Switzerland (Stucki, 2010). At each sampling location (D, U1, U2), benthic invertebrates were sampled in spring using a kick-net (25 cm × 25 cm opening, 500-μm mesh size). The same subsets of 12 sites were studied in 2013 and 2014 as in the water quality sampling. Sites were sampled between 13 March to 24 April 2013 and between 5 March to 11 April 2014, with lower elevation sites (m a.s.l.) sampled first (Stucki, 2010). The samples collected from each reach were pooled and stored in 80% ethanol prior to identification at a taxonomic level commonly used for biomonitoring in Switzerland (i.e., families for all taxa, but species level for EPT taxa (i.e., Ephemeroptera, Plecoptera and Trichoptera) using predefined identification literature (Stucki, 2010). Further details can be found in Burdon et al. (2016).

2.4.1. Invertebrate community descriptors

To describe invertebrate community variation, we focused on five diversity measures (i.e., Shannon Index, taxa and EPT family richness and their rarefied equivalents) and four trait-based indices (i.e., the Swiss IBCH, SPEAR Pesticides, Saprobic, and Sediment indices), as explained below. Calculations of these trait-based indices were based on the taxonomic resolution corresponding to the Swiss biomonitoring standard IBCH data (Stucki, 2010). We calculated these trait-based indices both including and excluding oligochaete worms (oligochaetes are known to feed on sludge particles (Ratsak and Verkuijlen, 2006)), because this taxon is strongly influential in community responses to wastewater inputs (Hynes, 1963; Burdon et al., 2016).

The Shannon Index (Shannon, 1948) is a measure of information entropy. The IBCH index is the French IBGN (*Indice Biologique Global Normalisé*) adapted for Switzerland (Stucki, 2010). It uses the presence of predefined indicator taxa in relation to total macroinvertebrate taxa richness to derive a score of stream health.

The SPEAR Pesticides Index (SPEAR Index hereafter) describes the proportion of taxa (%) susceptible to pesticides (Liess and von der Ohe, 2005; Knillmann et al., 2018). We used the SPEAR calculator 2018.05 as implemented in http://www.systemecology.eu/indicate/ (version, 1.0.0). The Saprobic Index describes shifts in the invertebrate community towards taxa that are more tolerant of low oxygen conditions, typical for high organic load situations (Bunzel et al., 2013). The Sediment Index (also known as the Empirically-weighted Proportion of Sediment-sensitive Invertebrates index; E-PSI) calculates the proportion of sediment-sensitive taxa in a community and was calculated using modified values from (Turley et al., 2016). For more information on these indices and their calculation, see SI S3.

2.5. Data analysis

2.5.1. Differences between up- and downstream locations

The χ^2 test was used to test for differences in the distribution across assessment classes at the U and D locations for the different water-quality parameters. Differences between U1 and D (non-impacted versus impacted site) were compared to differences between U1 and U2 (non-impacted in-stream controls) by paired *t*-tests. To quantify the effect sizes of the U1-D differences compared to the upstream variability (U1-U2), Cohen's d (Cohen, 1992) was calculated according to:

$$\mathrm{d} = \frac{\mu_D - \mu_{U1}}{\sqrt{\frac{(n_D-1)\times s_D^2 + (n_{U1}-1)\times s_{U1}^2}{n_D + n_{U1} - 2}}} \quad (4)$$

with $n_{D,U1}$ the number of observations at D and U1, respectively; $s_{D,U1}$ the standard deviation at D and U1, respectively; $\mu_{D,U1}$ the mean at D and U1, respectively. Because of some differences in the sampling strategies for some variables, the specific implementation was adapted as described in the SI. For further details on effect sizes, see SI S3. All analyses were conducted in R, version R-3.3.3 (R Development Core Team, 2013).

2.5.1.1. Variation partitioning (VP) models. We used constrained ordination techniques in variation partitioning models. The response variable in these VP analyses was derived from community abundance data with the taxonomic resolution used for biomonitoring in Switzerland. The Hellinger-transformed data used the Euclidean-distance metric in all models (Legendre and Gallagher, 2001). We analyzed each VP model with two sets of transformed community abundance data: one including and one excluding the oligochaeta, respectively. For predictor variables, we used data describing water quality (e.g., nutrients, TUs for MPs and for HMs, and general water chemistry parameters), habitat characteristics (see Table S3), upstream catchment properties (Table S1), and spatial location. To help normalize data and improve homoscedasticity, we log-transformed chemical and habitat data, and logit-transformed proportion data (e.g., % land cover of different land-use types). Data was then standardized using the 'decostand' function in R (i.e., centred on the column means and scaled by unit variance).

To avoid over-parameterising the VP models, we first removed colinear variables ($r < 0.75$). Following this initial step, we used a forward-selection procedure to select a subset of explanatory variables following the method recommended by Blanchet et al. (2008). Additionally, we included three groups of chemicals (HMs, pesticides, other MPs) as predictors to evaluate their relative importance. Response and predictor data sets included all sites and the locations U1 and D, except for the omission of one site (Val-de-Ruz) due to the detection of an unknown WW input upstream after data collection (U. Schönenberger, pers. obs.).

Spatial structuring of communities using Cartesian coordinates was assessed using Principal Coordinates of Neighbours Matrices (PCNM) analysis (Borcard and Legendre, 2002). PCNM descriptors (or axes) represent a spectral decomposition of the spatial relationships among the study sites (Borcard and Legendre, 2002). To avoid characterizing intra-sites differences, we used the coordinates of U1 for all three locations at each study site.

For these analyses, we used the R library "vegan" (Oksanen et al., 2010) with functions 'rda' (redundancy analysis) and 'varpart' (variation partitioning). The significance of each independent variation component was permutation-tested using 1000 randomizations (Peres-Neto et al., 2003). Results were visualised using the 'venneuler' package in R.

2.5.2. Principal component analysis (PCA)

To reduce the number of descriptors for general water chemistry and habitat, PCAs were run with the two sets of variables. For water chemistry, we selected 11 (electrical conductivity, pH, alkalinity, K^+, Ca^{2+}, Mg^+, Cl^-, SO_4^{2-}, TOC, silica, suspended solids) and for habitat characteristics 10 (variability of stream width, streambed modifications, depth variability, modification of the river bank, width of the river bank, bank quality, presence of mud, coverage with macrophytes, total suspendible solids, river depth) variables. The qualitative habitat descriptors were converted to numerical values between 0 and 1. A value of zero would correspond to fully artificial and a value of one to fully natural conditions. The full description of the translation of the qualitative field protocols into numerical values in provided in Table S3.

The values of the different variables were centered and scaled for calculating the PCA using R ("prcomp").

2.5.3. Structural equation modelling

To better understand the causal pathways leading to changes in macroinvertebrate communities, we used piecewise structural equation modelling (SEM) (Lefcheck, 2016). We used the r package 'piecewiseSEM' to perform path analysis with random effects (Lefcheck, 2016). The variable selection was based on the previous analysis of which factors influenced the upstream conditions and which ones had an impact on the change of the macroinvertebrate community between U and D (see Result section).

3. Results

3.1. Water quality

At the upstream locations, most of the nutrients levels corresponded to a very good or good status (Fig. S10) according to the Swiss river assessment system (Liechti, 2010). Based on soluble reactive phosphorous (SRP) and dissolved organic carbon (DOC), about 20% of the sites were classified as moderate or worse, however. For nitrogen species and phosphorous, the distribution of the quality assessment across sites was significantly shifted towards lower quality at D locations ($p < 0.0005$; χ^2 test, Fig. S10).

The impact of WW discharge on water quality was also clearly visible from huge effects sizes expressed as Cohen's d (according to Sawilowsky, 2009) of differences between U and D locations and *t*-test results comparing the variability between U1 and U2 locations and the U1-D differences (Table 1). Regarding organic MPs, the most pronounced increases downstream were observed for pharmaceuticals and household chemicals (see also Munz et al., 2017). For other groups, such as pesticides and HMs, the increase was less. HMs resulted in the highest values for toxic units (TUs) at both locations and exceeded those of MPs by at least one order of magnitude (Fig. S3).

Table 1
Differences between upstream and downstream conditions. Water chemistry describes dry-weather conditions; Cohen's d corresponds to median values across sites. *t*-Test compare the upstream-downstream differences to differences between the two upstream controls. Statistically significant (*p-values* < 0.05) differences are highlighted in bold. NA = not available because only one U location was sampled. Details for calculating Cohen's d can be found in the SI (S3, method description M5).

Variable group	Variable	Cohen's d	*p-values* (*t*-test)
General water chemistry	**Electrical conductivity**	30.7	0.003
	pH	−2.8	0.737
	Cl^-	28.1	0.001
Nutrients	**SRP**	45.5	0.005
	Nitrate	46.7	0.003
	NH_4^+	11.4	0.060
	DOC	7.1	0.021
Heavy metals (concentrations)	Co_{filt}	3.3	NA
	Cu_{filt}	1.5	NA
	Zn_{filt}	3.3	NA
Micropollutants	Pesticides (mean)[a]	0.6	NA
	Biocides (mean)[a]	3.4	NA
	Pharmaceuticals (mean)[a]	26.8	NA
	Corrosion inhibitors (mean)[a]	82.0	NA
Macroinvertebrates	IBCH index	NA[b]	0.058
	SPEAR index	−1.02	0.003
	SPEAR no oligo	−0.64	0.010
	Saprobic index	11.9	< 0.001
	Saprobic no oligo	1.1	0.016
	Sediment index	−0.2	0.400
	Sediment no oligo	−0.1	0.362
	Shannon div. EPT	−1.1	0.129
	EPT taxa rarefied	−0.5	0.081
	Taxa rarefied IBCH	−2.2	0.006

[a] Mean values calculated for median values per single compound across sites.
[b] Cohen's d values could not be calculated for all sites because standard deviations at some of the upstream sites were zero.

Despite the generally lower water quality at the downstream locations, the absolute nutrient levels demonstrated that the WWTPs performed generally well according to the regulatory requirements (data not shown). There was no indication of oxygen limitation downstream of the WWTPs based on the in-stream oxygen data (Fig. S2). Oxygen levels were well correlated between U and D, and decreased only moderately downstream (by 0.9 mg O_2 L^{-1}, on average, with 0.77 for the 2013 sites and 1.08 mg O_2 L^{-1} for the 2014 sites). Four sites had elevated NH_4^+ levels because of the absence of a nitrification step on the WWTP (Fig. S10). However, phosphorous removal was not very effective at several WWTPs causing poor quality for this parameter at many D locations (Fig. S10).

Water quality was also clearly affected by upstream land use. The most prominent influence was observed for nitrate, which showed a clear correlation with the percentage of arable land (Pearson correlation, $p < 0.001$; $R^2 = 0.89$). To a lesser degree this held also true for pesticides. The TUs of these group of chemicals increased with an increasing fraction of arable land in the catchment (Pearson correlation, $R^2 = 0.39$, $p = 0.0012$, Fig. S12).

3.2. Invertebrate communities

A total of 85 macroinvertebrate taxa were recorded from all sampling locations. Macroinvertebrate communities at U1 and U2 were generally dominated by chironomid dipterans, baetid mayflies, and gammarid amphipods, whereas oligochaete worms were highly abundant at D locations.

3.2.1. Variation partitioning of determinants of community structure

We used variation partitioning (models VP1–12, see Table 2, Table S4) to better understand the environmental and spatial drivers shaping macroinvertebrate community composition. We differentiated between factors representing "natural"[3] conditions (e.g., catchment size or Ca^{2+} concentrations), spatial relationships between sites, and finally factors reflecting human impacts (i.e., land use impacts on water quality, and/or habitat modifications).

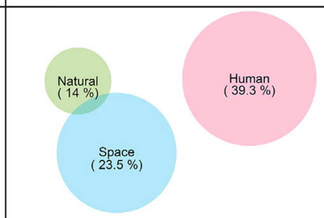
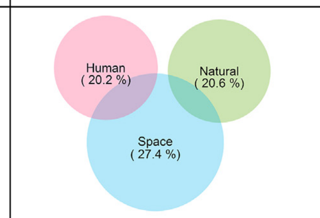
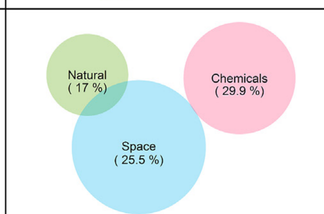
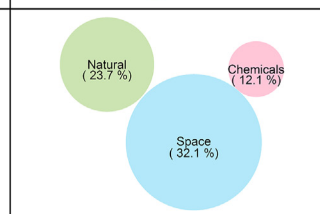
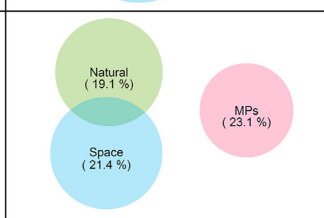
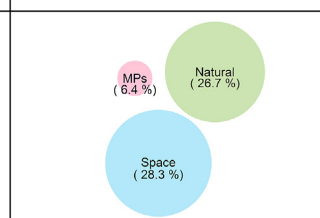
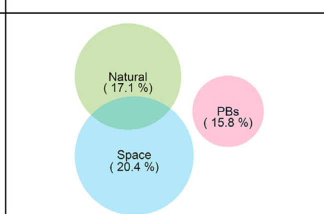
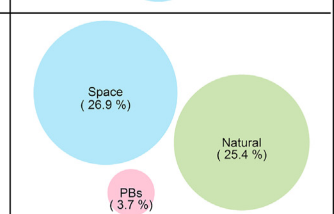
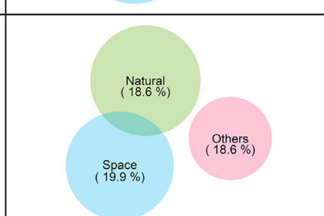
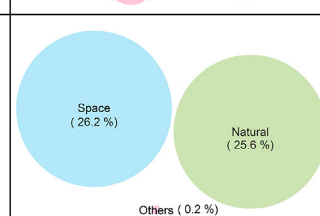
About 68% of the community composition was statistically explained by model VP1 when natural, human and space factors were considered jointly (both when oligochaetes were included and excluded). The relative influence of human impact was, however, substantially larger (39% explained variation) when oligochaetes were included, whereas only about 20% of the community composition was explained by human impact when oligochaetes were excluded (Table 2).

Studying separately the different factors reflecting human impact (i.e. water quality, land use) demonstrated that water quality explained the largest fraction (29.9% considering oligochaetes, and 12.1% excluding them). Impacts on habitat quality were always second (13.3% and 6.1%, with and without oligochaetes, respectively), while land use only exerted a statistically significant influence when oligochaetes were excluded from the analyses. The opposite held true for the fraction of wastewater, which only was relevant when oligochaetes were included in the analysis (see Table S4).

Human impacts on water quality affected nutrient levels, toxic pressure (expressed in TUs) as well as the concentrations of ions (e.g. Na^+ or K^+) which change due to fertilisation or wastewater discharge. Models distinguishing effects of nutrients and organic MPs revealed that nutrients only explained a significant fraction (3.6%) when oligochaetes were included in the community analyses. Without oligochaetes, nutrients did not have a statistical influence. Organic MPs however, explained significant fractions of the community composition in both cases (explaining 3.2 and 5.2%, respectively). A similar result was obtained

[3] "Natural"implies here without human impact *at the scale of the respective watersheds*. Due to global warming human impact may also shift biogeochemical equilibria for example.

Table 2
Results from variation partitioning models explaining the percentage of variation in macroinvertebrate community composition attributable to different predictor groups. Models were tested using macroinvertebrate community relative abundance data including and excluding the oligochaets. The factor *Natural* comprised catchment area, altitude, river width, coverage by moss, alkalinity and the concentration levels of Ca^{2+} and Mg^{+}. *Space* consisted of 7 PCNM axes. The full factor *Human* comprised wastewater dilution at Q_{347}; nutrient concentration (TN, TP, $NH4^{+}$), toxic pressure (TUs, toxic units) of MPs (pesticides, others) and concentrations of the two cations Na^{+} and K^{+}. Numbers indicate the percentage variation explained by the specific descriptor, i.e. without additional intersections independently. Note that values < 0 (possible for adjusted R^2 values) are not shown. Therefore, the numbers in the Venn-Euler diagrams do not necessarily add up to the respective total explained variation indicated. Venn-Euler diagrams are an *approximate* representation of model results. Further details are provided in Table S4, which reports the results of all 12 VP models.
* *P* < 0.05.

Model	Oligochaetes included		Oligochaetes excluded	
	Variation (adjusted R^2 %)	Euler-Venn diagram	Variation (adjusted R^2 %)	Euler-Venn diagram
VP1	68.1		68.7	
VP2	58.7		60.6	
VP7	51.9		54.9	
VP8	44.5		52.2	
VP9	47.4		48.7	

for pesticides only, which are part of the organic MPs (2.4 and 3.4%, respectively). The toxic pressure by HMs did not reveal any statistical relationship with the macroinvertebrate community (see Table S4). This indicates a limited impact of these chemicals on community composition but this effect may in part be explained by data being limited to the 2014 sites.

It is important to note that the variation explained by specific groups of chemicals (e.g. organic MPs, pesticides or nutrients) in models that explicitly accounted for two groups of chemicals was always substantially smaller than the percentage obtained when only one group of chemicals were considered separately (see Table S4). This demonstrates the difficulty to tease apart the respective roles of these chemicals based only on observational data in catchments where the pollutants have similar sources.

3.2.2. Upstream – downstream comparisons

The Swiss IBCH index showed no significant differences in stream health between U and D locations (Fig. S10). Approximately two thirds of the locations achieved a 'good' to 'very good' status. The remainder was predominantly rated as 'moderate', and only two locations at Colombier were classified as 'poor'.

More specific community metrics (e.g. trait based indices), however, showed clear differences between the U and D locations. The most pronounced effect was seen for the Saprobic index. Despite the presence of well-functioning WWTPs, the typical response to discharge of organic matter was highly significant and caused a pronounced increase of this index downstream of the WWTPs (Cohen's d = 11.9, paired *t*-test: $p < 0.001$, Table 1). This was mainly due to the strong increase of oligochaetes at the D locations. Excluding the oligochaetes from the analysis yielded a much reduced but still statistically significant effect (Cohen's d = 1.1, paired *t*-test: $p = 0.016$; Table 1). Surprisingly, no influence of the fraction of WW in the stream could be seen on the Saprobic index (Fig. 2). None of the water quality parameters, such as nutrients or TUs, improved the explanatory power of the observed difference between the U and D locations.

Also the SPEAR index changed from the U to the D locations. With or without considering oligochaetes, the SPEAR index indicated a significant ($p = 0.003$ and $p = 0.01$, respectively, paired *t*-test) loss of pesticide-sensitive species at D locations (Table 1). There was a trend for a stronger decline the higher the fraction of WW downstream (Fig. S11). The degree of statistical significance varied depending on the inclusion (Pearson correlation; $p = 0.049$) or exclusion ($p = 0.031$) of an outlier site (Hornussen, see Fig. S11) but was consistent across the 2013 and 2014 sites.

No other changes in the community composition were statistically significant beyond those that were direct consequences of the abundance of oligochaetes worms (e.g., affecting Shannon diversity for all IBCH taxa, Table 2).

We hypothesized that the response of the communities to wastewater discharge was larger the less impacted the U community (see also Burdon et al., 2016). This expectation was partially fulfilled. For example, when considering the Saprobic index including the oligochaetes there was a clear negative trend between U and the change to D (Fig. 2). A similar trend was also observed for the SPEAR index (Fig. 2).

Except for the Saprobic index including oligochaetes, the three indices (Saprobic, SPEAR, Sediment) at D were similar to the values at respective U locations (Fig. 3). This indicates that the factors controlling the composition at U had also a strong impact at D.

Given the relevance of the upstream status of the macroinvertebrate community for the response to wastewater and for the downstream conditions in general, we also analyzed the potential factors (land use, habitat, water quality) influencing the SPEAR and the Saprobic index at the upstream locations.

In contrast to the VP analyses of the overall community composition, there was a strong influence of land use, specifically intensive agriculture, on the trait-based community descriptors (Fig. S13). The SPEAR index was decreased with the increasing fraction of arable cropping in the upstream catchment (Pearson correlation, $p < 0.001$, $R^2 = 0.45$), whereas the Saprobic index increased with increasing fraction of arable land and pastures (e.g., meadows; Pearson correlation, $p = 0.049$, $R^2 = 0.17$).

3.2.3. Structural equation modelling (SEM) of the Saprobic and SPEAR indices downstream

The findings for the SPEAR and Saprobic indices can be summarised in structural equation models. The respective path models (Fig. 3) and the related statistics (Table S5) demonstrate commonalities but also clear differences regarding the factors influencing these two indices. For both indices, the extent of intensive agriculture was a dominant driver of the upstream conditions and communities (Fig. 3, Fig. S13) and hence, indirectly for those at location D. While the upstream SPEAR values were strongly “directly” (statistically) related to values downstream (Fig. 2, Fig. 3), the upstream Saprobic index was only indirectly related to the Saprobic index at location D. The upstream Saprobic value influenced the degree by which this index changed between the U and D locations (Fig. 1, Fig. 3), whereas WW played a much smaller role. While we found no relationship of the Saprobic index and any of the WW descriptors (amount, quality), the fraction of WW had an impact on the SPEAR index when the amount of suspendible sediment in the stream bed was jointly considered (Fig. 3, Table S5). This trend was statistically significant if one outlier site (Hornussen) was removed (Fig. S16). Overall, the emerging patterns and statistical relationships were consistent across the 2013 and 2014 sites when analyzed independently (see Table S5, Figs. S15) supporting the robustness of the findings.

Despite the large number of explanatory variables considered in this study, the resulting path models are very simple. Note that in more complex models, the explained total variance did not increase and single variables did not yield significant results. The TUs of HMs, for example, showed a weak, significant correlation with the SPEAR index across all locations. However, in a multiple regression including other chemicals, such as nitrate or TUs for pesticides, the influence of HMs was not detectable.

4. Discussion

Chemical pollution is a pervasive threat to the biodiversity and ecosystem services provided by aquatic systems (Vörösmarty et al., 2010). In many anthropogenically-influenced catchments, treated WW can be a major source of pollution with strong impacts on receiving habitats (e.g., Münze et al. (2017)). However, the strength of wastewater impacts may depend on the magnitude of co-occurring pressures (e.g., upstream land-uses) and biological factors, such as dispersal (Burdon et al., 2016). Using data from 23 Swiss streams (one site had to be excluded from the analysis, see above), we found that the effect of WW (dilution and composition) on community composition was strongly mediated by the intensity of agriculture in the upstream catchment (see Fig. 3). Specifically, the response to treated wastewater weakened as the intensity of agricultural land-uses increased upstream. There was a strong “noise to signal ratio” for most invertebrate community responses to wastewater, pointing to a strong effect of regional and

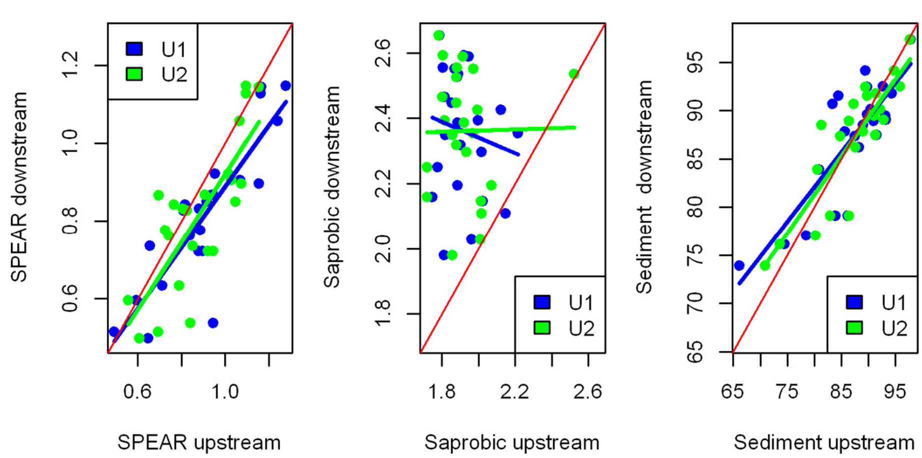


Fig. 2. Relationships between the three indices (SPEAR, Saprobic and Sediment) at the downstream locations as a function of the upstream values. The blue and green lines represent the Pearson correlations, the red line the 1:1 line (SPEAR index: adjusted R^2: 0.732 and 0.603, *p*-values < 0.001 for U1 and U2; Saprobic index: adjusted R^2: −0.02 and −0.047, *p*-values = 0.482 and 0.949 for U1 and U2, respectively; Sediment index: adjusted R^2: 0.666 and 0.741, p-values < 0.001 for U1 and U2).

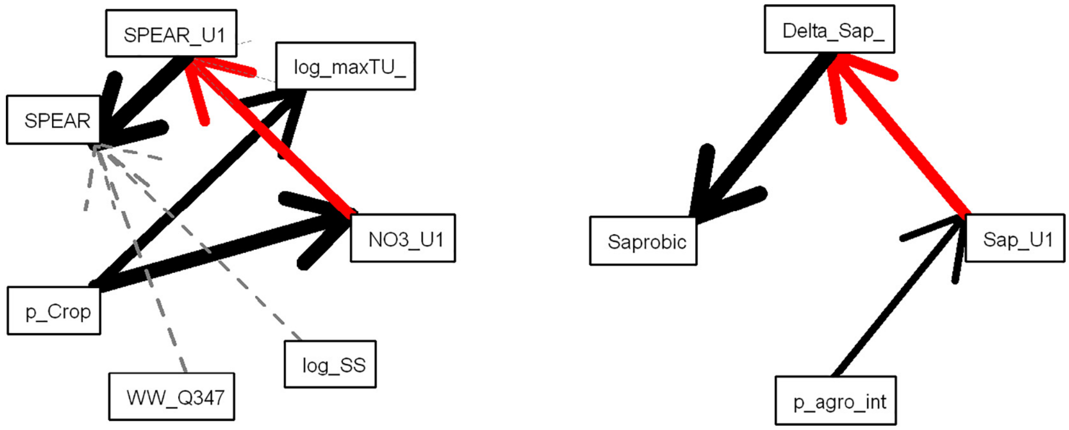


Fig. 3. Path models depicting the identified relationships between influencing factors and the SPEAR (left) and the Saprobic index (right) including the oligochaetes at the downstream locations. Red arrows indicate a negative linear relationship, black arrows positive linear relationships. The line width represents the statistical significance (see Table S5 for more statistical details). Delta_Sap: Difference between Saprobic index at D and U1; log_maxTU: maximum sum of TUs for pesticides and biocides (logarithm), log_SS: total suspendible (benthic) sediment (logarithm), NO3_U1: nitrate concentration at U1, p_agro_int: areal fraction of arable cropping and pastures in the catchment, p_Crop: areal fraction of arable cropping in the catchment Saprobic: Saprobic index at D, Sap_U1: Saprobix index at U1, SPEAR: SPEAR index at D, SPEAR_U1: SPEAR index at U1, WW_Q347: fraction of WW under low flow conditions.

catchment-wide influences compared to the local impacts of this additional pressure.

4.1. The relevance of micropollutants

Water quality overall was confirmed as an important driver of macroinvertebrate community composition (see Table 2) and the VP analyses puts its relevance into the context of other environmental factors, such as habitat modifications or land use. Similar to findings by Rico et al. (2016) in the Danube river, where habitat and general water chemistry were major explanatory factors, we observed that toxicants (MPs, HMs) explained a significant but low percentage of the community variability. Much of this was attributable to the toxic effects of pesticides (Table 2, VP7 to VP12). However, the statistical analyses also showed the difficulty to separate the relevance of single influence factors in such an observational study despite the decent sample size.

It was notable in our study that agricultural land-uses (e.g., arable cropping) had a strong influence on specific community metrics such as the SPEAR index, whereas the local impacts of WW were relatively weak (Fig. 3). Comparing the extent of change of this index related to arable cropping (Fig. S13, delta SPEAR $\approx$ 25 units for arable land ranging between 0% and 80%) with the trend for change due to WW (Fig. S11, delta SPEAR $\approx$ 5 units for WW flow contribution ranging between 0% and 60%) suggests that agriculture was about 4 to 5 timesmore influential than WW discharge. The opposite trend was observed for the Saprobic index, for which WW seems to be about twice as influential compared to agriculture compared to effects size (see Fig. S11, S13). The weak WW impact on the SPEAR index is in contrast to recent findings from Germany (Münze et al., 2017), where WW decreased the index on average by 40%.

Interestingly, the strongest relationship between biological responses (e.g., the SPEAR index) and water quality was observed using nitrate concentrations. This result confirms that the extent of arable cropping and other intensive agricultural land uses can strongly influence stream invertebrate communities (Kaelin and Altermatt, 2016; Ryo et al., 2018). At the concentration levels observed, however (i.e., the mean range of upstream sampling locations was 0.8–6.5 mg L^{-1}), nitrate would be expected to have no or only weak direct effects on the majority of macroinvertebrates (Camargo et al., 2005). This suggests that nitrate was a good proxy for other co-occurring stressors (i.e., pesticides), which we did not (fully) capture by our sampling and analytical approach. For example, despite the broad range of compounds considered in our study, important groups such as pyrethroid pesticides (Moschet et al., 2014a; Antwi and Reddy, 2015) were not included. These chemicals are highly toxic to invertebrates, but were beyond the scope of our study because of their often very low concentrations (i.e., picogram per liter range) requiring highly specialized analytical procedures (Moschet et al., 2014a).

Additionally, episodic, rain-driven concentration peaks (e.g., of pesticides, sediment) have not been sampled in this study. While our results clearly demonstrate a decline in water quality below WWTPs (Table 2), they reflect dry weather conditions. Episodic pesticide peaks due to diffuse losses from agriculture can induce high toxic pressure (Liess and Schulz, 1999; Spycher et al., 2018) and often dominate over inputs from urban sources (Moschet et al., 2014b). Such events affect U and D locations similarly, thus reducing the local impacts of WW-induced changes in water quality. This observational bias may also have affected the relative weight for different environmental factors in the VP models. It is conceivable that the relevance of MPs and specifically pesticides (VP4, 8) would increase upon a more comprehensive (temporal) quantification of these chemicals. In the current study their effects may have been partially attributed to nutrients (see VP6, 10) because nitrate seems to have been a good proxy for the influence of arable land (Fig. 3, see above).

While pesticides had a clear effect on the macroinvertebrates, HMs did not show any effect despite their much higher TU values. This may be due to the fact that some highly toxic pesticides were not measured (see above) but may also point to a potential discrepancy between ecotoxicological assessment and the observed biological effects (i.e., the relevance of HMs compared to organic pollutants such as pesticides). A recent study for example (Liess et al., 2017) suggests that community effects of HMs only occur at TUs far above what is known for pesticides. It has also to be considered that we used acute EC_{50} as a measure for all toxicants irrespective of their mode of action (e.g., acute versus chronic). This procedure was chosen because only acute EC_{50} values were available for a sufficient number of compounds.

4.2. Detecting change: local versus regional factors

We implicitly assumed with the sampling design that we would be able to detect strong effects of environmental filtering (i.e., species sorting; (Heino, 2013)) on invertebrate communities due to WWTP pollution. However, metacommunity theory has emphasized that community structure is determined not only by local abiotic environmental conditions (i.e., environmental filtering leading to species sorting), but also by biotic interactions and dispersal (Heino, 2013). In particular, mass effects (i.e., the presence of species in environmentally suboptimal sites due to high dispersal rates from environmentally suitable sites) may obscure the impacts of local stressors, because dispersal from source sites allows persistence at impacted low quality sites. In our study, mass effects from upstream sites may have partially masked the true extent of WW impacts because of the short distances between the U and D locations (approx. 200–600 m). However, non-insect stream invertebrates such as gammarid amphipods are typically slow to recover from chemical disturbances (Thompson et al., 2016). This makes it difficult for dispersal-related processes to completely compensate for the regular losses of organisms due to persistent WW pollution. Furthermore, Münze et al. (2017) observed an average SPEAR index decrease by 40% downstream of WWTPs despite shorter (50 m) distances below the discharge points.

Another potential factor influencing our ability to fully detect changes due to WW impacts (and associated stressors) was the taxonomic resolution used for macroinvertebrate community data. We used a relatively coarse level of taxonomic resolution as applied in the standard methods for Swiss river health assessment (Stucki, 2010). This may have hampered our ability to detect relationships between environmental conditions and community composition. For example, highly-resolved taxonomic identification using DNA metabarcoding revealed effects of multiple stressors on chironomid midge communities that were not detected at the family-level (Beermann et al., 2018). As several taxa (e.g. chironomids and oligochaeta) were only assigned to a single taxon in our study, there were likely undetected responses regarding the specificity of different chemicals and environmental factors at this coarse taxonomic resolution (Andujar et al., 2018).

Finally, other ecological (i.e., trophic and non-trophic interactions) and evolutionary processes may also have influenced the results. Biotic interactions can alter the outcome of stressors and may enable species persistence if antagonistic interactions become beneficial (e.g., through facilitation) (Fugère et al., 2012). Likewise, the stress tolerance of exposed populations can increase due to physiological acclimation or adaptive genetic responses (Reznick and Ghalambor, 2001) – although the latter might be prohibited due to high gene flow (Rolshausen et al., 2015) among U and D locations. Furthermore, species interactions might also dampen adaptive responses (Becker and Liess, 2017). However, inferring the relative role of these multiple influencing factors requires targeted experimental and genetic studies with adequate taxonomic resolution.

5. Conclusions

Our findings suggest that water quality is a major factor shaping macroinvertebrate communities in headwater streams. Impairment of water quality causing a deterioration of the macroinvertebrate communities seem to be strongly linked to intensive agriculture, specifically to arable land and other crops with intensive pesticide use. In contrast, WW effects seem to be less relevant except for the occurrence of oligochaetes.

However, before drawing unequivocal conclusions about the limited impact of WW on macroinvertebrate communities in streams, current gaps in understanding their responses to chemical stressors need to be considered. Gaps identified or confirmed by our study relate to the quantification of the chemical exposure on one hand and to the biological response on the other hand. Improved understanding of macroinvertebrate responses to chemical stress requires an increase in the extent of spatio-temporal sampling of chemical and biological variables as well as the taxonomic resolution of indicator biota. The implementation of technological advances, such as DNA metabarcoding and/or online analysis of MPs, would greatly improve our understanding of how chemical contaminants affect ecological and evolutionary processes. Armed with this knowledge, we would be better placed to assess the risk posed by these compounds in the real world, thus supporting improved management of aquatic resources.

CRediT authorship contribution statement

F.J. Burdon: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **N.A. Munz:** Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing. **M. Reyes:** Investigation, Resources, Data curation, Writing - review & editing. **A. Focks:** Methodology, Investigation, Resources. **A. Joss:** Conceptualization, Resources. **K. Räsänen:** Conceptualization, Methodology, Writing - review & editing, Supervision. **F. Altermatt:** Conceptualization, Methodology, Writing - review & editing. **R.I.L. Eggen:** Conceptualization, Resources, Funding acquisition, Project administration, Supervision. **C. Stamm:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Funding acquisition, Project administration, Supervision, Visualization.

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Supplementary data

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