CHAPTER FOUR

Unravelling the Impacts of Micropollutants in Aquatic Ecosystems: Interdisciplinary Studies at the Interface of Large-Scale Ecology

C. Stamm[*,1], K. Räsänen[*], F.J. Burdon[*], F. Altermatt[*,†], J. Jokela[*,‡], A. Joss[*], M. Ackermann[*,§], R.I.L. Eggen[*,¶]

[*]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[†]University of Zurich, Zürich, Switzerland
[‡]ETH Zürich, Institute of Integrative Biology, Zürich, Switzerland
[§]ETH Zürich, Environmental Systems Science, Zürich, Switzerland
[¶]ETH Zürich, Institute of Biogeochemistry and Pollutant Dynamics, Zürich, Switzerland
[1]Corresponding author: e-mail address: christian.stamm@eawag.ch

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Abstract

Human-induced environmental changes are causing major shifts in ecosystems around the globe. To support environmental management, scientific research has to infer both general trends and context dependency in these shifts at global and local scales. Combining replicated *real-world experiments*, which take advantage of implemented mitigation measures or other forms of human impact, with *research-led* experimental manipulations can provide powerful scientific tools for inferring causal drivers of ecological change and the generality of their effects. Additionally, combining these two approaches can facilitate communication with stakeholders involved in implementing

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management strategies. We demonstrate such an integrative approach using the case study *EcoImpact*, which aims at empirically unravelling the impacts of wastewater-born micropollutants on aquatic ecosystems.

1. LARGE-SCALE ECOLOGY AND HUMAN IMPACTS ON ECOSYSTEMS

Rapid environmental change during the 'anthropocene' is a global phenomenon that has substantially altered terrestrial and aquatic ecosystems (Emmerson et al., 2016; Gray et al., 2016; Lewis and Maslin, 2015). These anthropogenic impacts range from global climate change and habitat fragmentation to invasive species and pollution (Millennium Ecosystem Assessment, 2005). To help support appropriate management decisions in face of such a multitude of large-scale changes, a good mechanistic understanding of how ecosystems respond to external drivers is important (Evans et al., 2013; Rockström et al., 2009). However, predicting the response of complex ecological systems to environmental change (natural or anthropogenic) is not trivial (Mouquet et al., 2015).

A key challenge in this context arises from several simultaneously acting and potentially interacting factors that can lead to multiple-stress situations (Tockner et al., 2010). This holds true at different spatial scales, from global (Vörösmarty et al., 2010) and regional (Hering et al., 2012) to local (Robinson et al., 2014). Describing patterns of ecosystem change is easier than understanding the causal drivers—not to mention being able to predict future ecological change (Burkhardt-Holm et al., 2005). Predicting the future state of ecosystems requires an ability to disentangle the effects of single factors from their possible multifactorial interactions, and a mechanistic understanding across all scales of observation (Evans et al., 2013). As a potential solution to this conundrum, the concept of the 'ecological forecast horizon' has been proposed as a quantitative link between the needed forecast quality and the actual predictability of ecological variables (Petchey et al., 2015). This then raises the question: which level of detail is necessary to make reliable predictions at different spatial and temporal scales?

From a management perspective, large-scale predictions are generally necessary to support policy changes that concern entire regions, countries or continents (e.g. how to preserve biodiversity and/or ecosystem services (Durance et al., 2016)). More detailed local scale models may be required to justify a particular management decisions with local effects, such as how to

modify stream morphology to achieve an optimum for societal needs (Hostmann et al., 2005).

The issue of how much detail is needed for sound management decisions—and at which spatial scales—can be illustrated by the example of river restoration (Palmer, 2010). Restoration activities have often been triggered by ecological insight (Palmer et al., 2005). The ecological paradigm that 'loss of habitat diversity decreases biodiversity' was the foundation of many river restoration activities (Ward et al., 2002), whereby riverine biodiversity was predicted to improve if stream morphology was restored to a more natural state. As data from restoration projects accumulated, however, critical syntheses revealed that the expected success of restoration projects was achieved much less frequently than expected (Bernhardt and Palmer, 2011; Kail et al., 2015).

One of the main reasons for the low success rate of restoration activities was that the spatial scales of action were insufficient (Bernhardt and Palmer, 2011). Specifically, restoration has been typically undertaken at the reach or segment scale (Frissell et al., 1986), but it turned out that the paradigm 'diversity begets diversity' (MacArthur and MacArthur, 1961) was too simplistic. This is because habitat morphology is not the only important factor promoting local biodiversity (Sundermann et al., 2011). In particular, regional processes (including species distributions or water quality) are critically important for enabling establishment of organisms at restored habitat sites (Sundermann et al., 2011). These river restoration examples clearly illustrate that both regional and local drivers may be key predictors for ecological responses that should be considered in concert.

The problem of multiple stressors acting upon an ecosystem is also pertinent when dealing with ecological effects of water pollution, which is one of the major threats for biological diversity (Millennium Ecosystem Assessment, 2005). Water quality issues have a large-scale component, firstly because of the global distribution of the human population and hence the large number of streams that are affected by chemicals, and secondly due to the persistence of many chemicals in rivers and the long-range downstream effects of pollution (Ruff et al., 2015). On the other hand, as we will emphasize later, local variation in both chemical exposure and the ecosystem (e.g. source of the ecological community) may play a dominant role in how specific ecosystems respond.

In what follows, we show how the use of replicated, interdisciplinary *real-world experiments*, combined with targeted manipulative experiments to infer causality, can help to tackle questions on multiple stressor effects

in the context of large-scale ecology. We illustrate the approach with a case study that aims at unravelling the impacts of wastewater-borne micropollutants (MPs) on stream ecosystems. We focus on these chemicals because they are prevalent in treated wastewater (WW; see Boxes 1 and 2) that influence water quality of many surface waters of different characteristics. For understanding the ecological effects of MPs, it is therefore critical to

BOX 1 Treatment of Wastewater and the Removal of Micropollutants (MPs) in Wastewater Treatment Plants (WWTPs)

Since the 1950s, wastewater (WW) treatment started being systematically implemented in urban areas of developed countries to cope with the waste of the growing human population. Today's conventional treatment primarily targets the removal of (i) particulate matter to reduce the microbial and pathogen load, (ii) degradable organic compounds to reduce the oxygen depletion in receiving waters, (iii) nutrients (i.e. nitrogen and phosphorus) to avoid eutrophication, and (iv) specific macropollutants, such as ammonia and nitrite (Neumann et al., 2015). This is generally achieved via a sewage network conveying the wastewater to a centralized wastewater treatment plant (WWTP). The main goal of implementing the required sewer and WW treatment is to protect drinking water resources, to achieve bathing water quality in recreational areas and to protect the aquatic environment from ecological degradation.

In Switzerland more than 95% of the municipal WW undergoes treatment in centralized WWTPs. Effluent from such WWTPs is still enriched with nutrients, other pollutants and microorganisms that are all discharged as complex mixture into the receiving waters (Fig. 7). Accordingly, WW effluent can still be a major input of pollutants to water bodies. Additionally, storm water overflows discharge (diluted) untreated WW during larger rainfall events. Such overflows are built to keep infrastructure costs of WWTPs at an affordable level, but come at the risk of releasing pollutants into the environment.

The trend in today's legislation in Europe (e.g. EU water framework directive; http://ec.europa.eu/environment/water/water-framework/index_en.html), as well as in developing WW infrastructure, is to avoid negative impacts in general rather than simply comply with thresholds set for specific quality parameters. The implementation of MP removal is part of this trend (Box 3): ozonation and activated carbon filtration are two technical means for significantly reducing the load of many MPs in WW (Hollender et al., 2009). For the sake of cost effectiveness, these technologies are mostly employed as an additional final step after state-of-the-art biological WW treatment.

BOX 2 Micropollutants

Micropollutants (MPs) can be defined as anthropogenic chemicals that occur in the (aquatic) environment well above a (potential) natural background level due to human activities but with concentrations remaining at trace levels (i.e. up to the microgram per litre range) (Fig. B.1). Thus, MPs are defined by their anthropogenic origin and their occurrence at low concentrations. Thousands of chemicals fall into this category (Schwarzenbach et al., 2006) and hundreds of them have been found at *EcoImpact* sites. MPs can consist of purely synthetic chemicals, such as strongly halogenated molecules (e.g. fluorinated surfactants), or of natural compounds such as antibiotics (e.g. penicillines) or oestrogens. MPs may originate from a wide range of sources (e.g. agriculture, households, traffic networks or industries) and enter water bodies through diverse entry paths. Depending on the source, MP transfer occurs as diffuse (e.g. agricultural land use) or as point source pollution, for which (municipal) WWTPs are important examples (see Box 1).

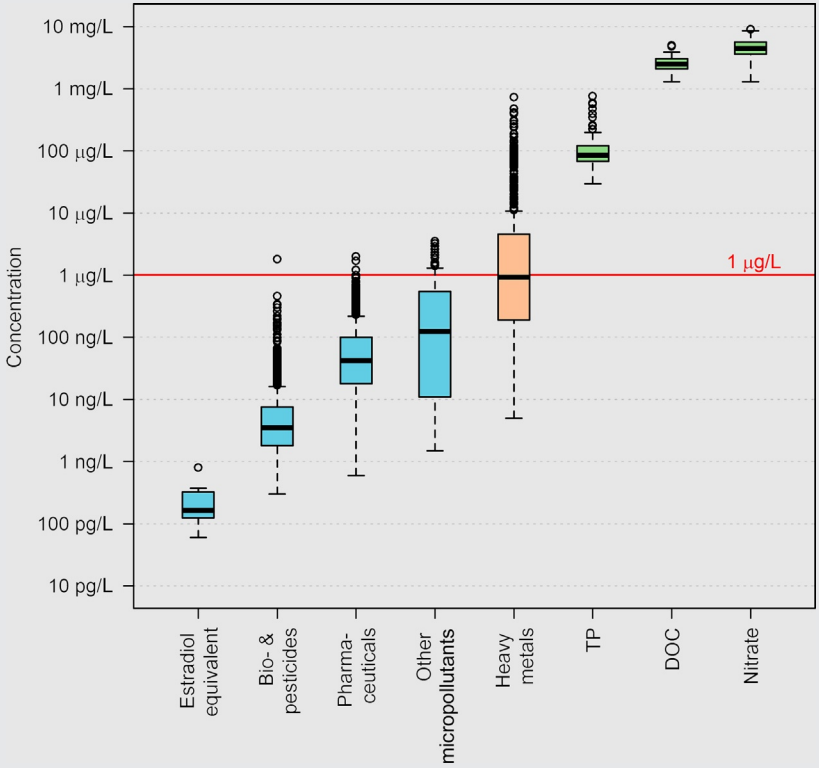

Fig. B.1 Boxplots of the ranges of measured pollutant concentrations (or effect concentrations for estradiol) illustrating the distinction between micropollutants (MPs, *blue* (*grey* in the print version)) and macropollutants (*green* (*light grey* in the print version)) across the 24 *EcoImpact* sites. Heavy metals (*red* (*grey* in the print version)) fall in-between the two classes. *DOC*, dissolved organic carbon; *TP*, total phosphorus. The *red* (*grey* in the print version) *line* indicates the concentration that is generally used to discriminate MPs from other pollutants. Box size corresponds to the first and third quartiles (25% and 75% quartiles); whiskers extend to the most extreme data point which is no more than 1.5 times the length of the box away from the box; *circles* depict outliers. The details of the methods are provided in Box 5.

Continued

BOX 2 Micropollutants—cont'd

The concentrations of MPs in aquatic environments generally make up only a tiny fraction of the dissolved organic matter content (see Fig. B.1). Why should we then be concerned about MPs? The answer to this is that generally these compounds are designed to exert very specific (biological) effects. These include curing a certain disease (e.g. bacterial infections), avoiding growth of unwanted weeds (e.g. herbicides), or providing a calorie free alternative to sugar (artificial sweeteners). In short, the biological activity of MPs is generally by orders of magnitude higher than that of average dissolved organic matter. How MPs act upon organisms—their mode of action—is often very specific and may predominantly affect specific groups of organisms (e.g. only vertebrates, only fungi, etc. (Fig. 2)). In this regard, MPs differ fundamentally from nutrients, which are essential to all organisms.

evaluate what general (large-scale) trends are, as opposed to environmentally contingent effects where local context dominates (Table 1).

1.1 Micropollutant (MP) Impacts at Different Levels of Biological Organization

Early on in aquatic ecology, it was recognized that the release of pollutants into streams can have acute and severe consequences for aquatic ecosystems (Hynes, 1963; Neumann et al., 2015). The effects of pollutants can range from singular catastrophic spills to the continuous release of contaminants at lower concentrations (Fig. 1). Catastrophic spills typically lead to concentrations causing acute toxic effects and capture the attention of both the general public and research communities. These events can trigger subsequent studies to quantify the extent and type of environmental damage and the recovery process (e.g. Güttinger and Stumm, 1992; Thompson et al., 2015).

The continuous release of chemicals, such as the discharge of untreated WW, can also cause negative effects that are easily discernible. For example, without treatment, WW discharges can cause eutrophication or fish kills (Hynes, 1963), but also outbreaks of human diseases caused by pathogens (Neumann et al., 2015). In developed countries, such effects have been successfully mitigated by the introduction of wastewater treatment plants (WWTPs) (Vaughan and Ormerod, 2012). WWTPs are designed to remove pathogens, nutrients, easily degradable organics and particulates (Box 1). However, toxic or highly biologically active chemicals are still discharged in substantial amounts (Petrie et al., 2015; Singer et al., 2016; Ternes,

Table 1 'The Big Question': *How Do MPs Affect Aquatic Ecosystems* from an Interdisciplinary Perspective

Research Fields	General Trends?	Context Dependency?	Key Approaches (as Exemplified from the Project *EcoImpact*)
Environmental chemistry	• How many MPs? • What kind of MPs? • At what concentrations do MPs occur?	• How do MPs vary locally depending on WWTP?	• Sampling of multiple sites with a different WWTP technology • Spatially and temporally replicated water 'spot' sampling • Passive samplers • Broad analytical window (high-resolution mass spectrometry)
Ecotoxicology	• Are MPs toxic? • What is the role of MP mixtures? • What are the cellular mechanisms of MP toxicity?	• How does MP toxicity depend on taxa? • How does MP toxicity differ between laboratory and field?	Lab and field assays of MPs with different modes of action (affecting algae, invertebrates and fish): • PICT assays → community tolerance of periphyton (algae, bacteria) • Gammarus feeding assays → indicator of toxicity • Fish gene expression → upregulation of detoxification genes
Ecology	How do MPs affect: • Population demography • Community diversity • Ecosystem function	• How do species diversity responses to MPs depend on landscape use? • How do MPs interact with nutrients, temperature and/or pathogens? • How do MP effects differ across tropic levels?	• Spatially and temporally replicated field surveys • Manipulative flume experiments (WW dilution or MP spiking) • eDNA analyses for microbial diversity • *Gammarus* size distribution • Species diversity indices (EPT, SPEAR, Saprobic Index) • Organic matter processing assays (leaf litter and cotton strips) • *Gammarus*–leaf litter assays • Ecosystem respiration

The table illustrates how 'large-scale' (general trends) and 'local scale' (context dependent) questions may be approached in different research fields. To address 'the big question', expertise and synthesis across fields are needed. In the context of *EcoImpact*, these questions are addressed combining two kinds of *real-world experiments* (upstream–downstream of WWTP (Fig. 3) and before–after (BACI) WWTP upgrade with field and flume experiments (Box 4).

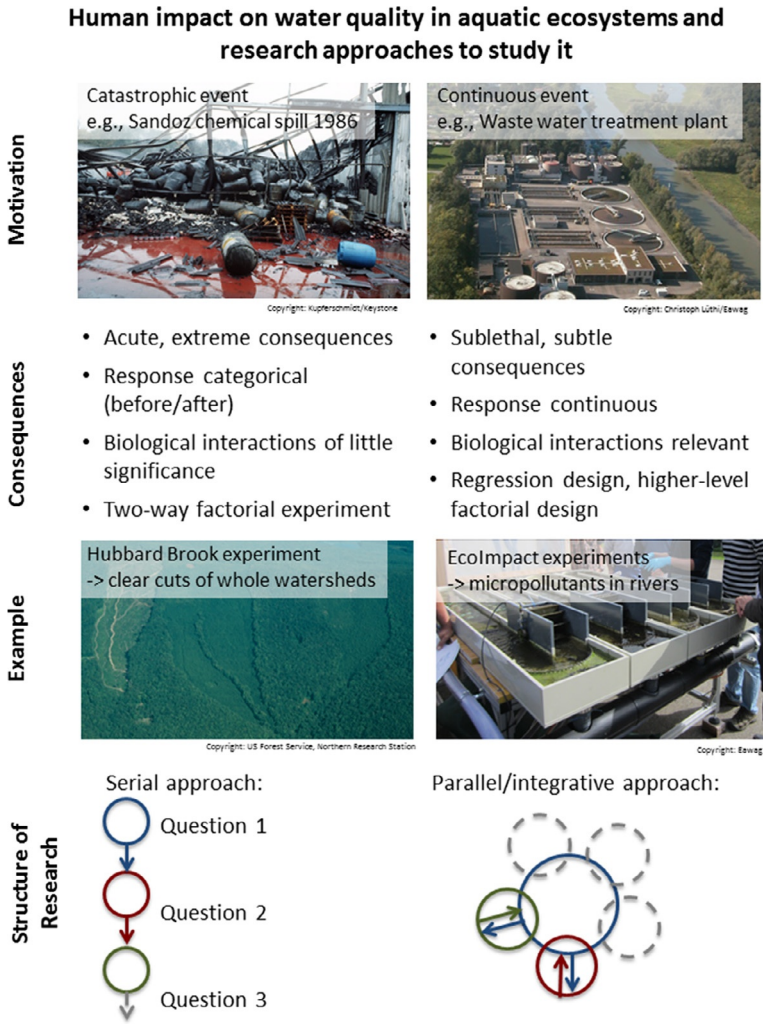

Fig. 1 Conceptual links between different types of environmental 'triggers' (catastrophic events vs continuous impacts) that reflect different types of human impacts on aquatic ecosystems, and how the type of impact may motivate research and project organization.

1998; Box 2). Moreover, the increasing use of chemicals in agriculture, households and industry is causing the number and amount of chemicals in WW effluent to increase across the globe (Keller et al., 2014).

Many of these compounds constitute MPs that occur frequently in high numbers (hundreds to thousands) but at low concentrations (Box 2; Schwarzenbach et al., 2006). This form of pollution is widespread, occurs

continuously over time and is much more commonplace than catastrophic events. Due to the widespread presence of wastewater-born MPs in aquatic environments (Kolpin et al., 2002) and the societal desire for pristine water resources, the ecological consequences of MPs in streams at all levels of biological organization have received considerable research attention over the last three decades (Brodin et al., 2013; Schwarzenbach et al., 2006; Ternes, 1998). Ecotoxicological tests have demonstrated the toxicity of a wide range of single MPs, MP mixtures (Carvalho et al., 2014; Silva et al., 2002) and treated WW effluents (Bundschuh and Schulz, 2011) on aquatic organisms.

These pollutants not only harm individual organisms, but also effects propagate to higher levels of biological organization. For example, at the population level, treated WW affected sex ratios of gammarid amphipods (Peschke et al., 2014). At the community level, pollution-induced community tolerance (PICT) experiments suggest that periphyton communities may acquire tolerance to MPs through environmental filtering, physiological acclimation and, potentially, evolutionary processes (Rotter et al., 2011; Tlili et al., 2015). Field surveys of macroinvertebrate communities indicate that pesticides from WWTPs may decrease the fraction of sensitive species through environmental filtering (Bunzel et al., 2013; Burdon et al., 2016). At the ecosystem level, pharmaceuticals can induce behavioural changes in fish leading to more intense predation on prey communities in aquatic food webs (Brodin et al., 2013; Heynen et al., 2016) and key processes, such as in-stream metabolism, can be affected in complex ways due to subsidy effects of nutrients and stress effects of MPs (Aristi et al., 2015; Rosi-Marshall et al., 2013).

During the last two decades, progress has been made in merging concepts from ecotoxicology and ecology to predict how chemical pollution might affect ecosystems (Fischer et al., 2013). Concepts like 'stress ecology' (Van den Brink, 2008; van Straalen, 2003) have been developed, there have been advances in assessing indirect MPs effects in food webs through behavioural ecology (Brodin et al., 2014), and community ecology has been demonstrated to allow for predictions how aquatic mesocosm communities respond to mixtures of different chemicals (Halstead et al., 2014).

Although these studies demonstrate the ecological relevance of (wastewater-born) MPs, predicting changes in entire food webs and ecosystems is still a major challenge because:

(1) *MPs from WWTPs generally occur at sublethal concentrations*. Due to their low concentrations in the environment, effects of exposure to these compounds may strongly depend on the ability of individual organisms

to physiologically acclimate and populations to adapt phenotypically (either through phenotypic plasticity or through genetic changes (Capy et al., 2000)). Realized effects may also be environmentally contingent, depending on resource availability, presence of other stressors (including other MPs), and food web interactions (see Fig. 2).

(2) *The occurrence of wastewater-born MPs is highly correlated with the input of other WW constituents*, particularly nutrients and microorganisms (Box 1). This highlights the problem of isolating MP effects when

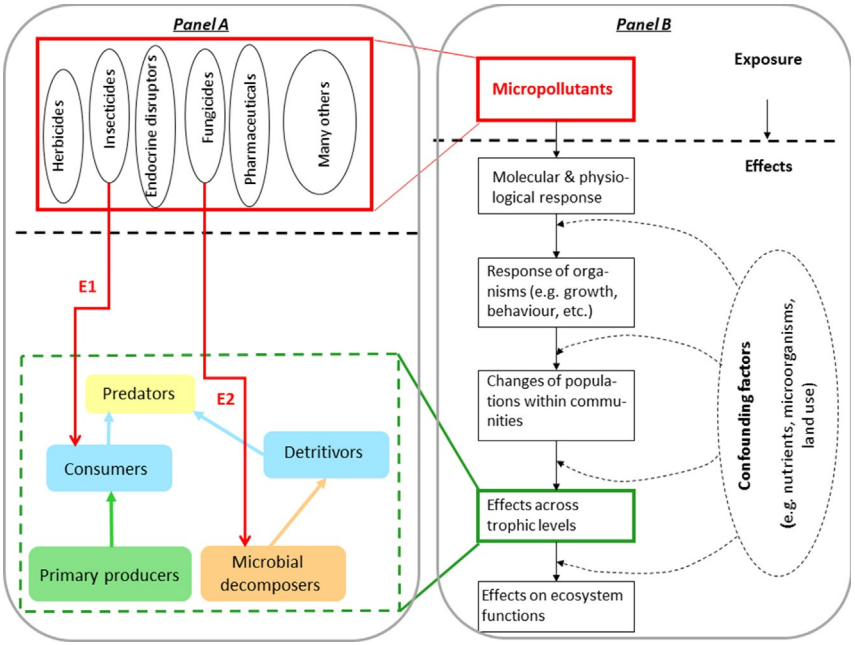

Fig. 2 Conceptual representation of how exposure to micropollutants (MPs) may cause effects in ecosystems at different levels of biological organization. MPs consist of diverse chemical groups (panel A, *red* (*grey* in the print version) *rectangle* at the top showing a selection of MPs) with specific modes of action that affect different compartments in a food web (exemplified with two *red* (*grey* in the print version) *arrows* (*E1*, *E2*) in panel A). The biological effects of MPs can reach different hierarchical levels (panel B) that are interlinked across a given food web and ecosystem. Simple predictions can be made for given types of MPs. For instance, if the key MPs consist of insecticides, then sensitive invertebrate species may disappear from the community (*arrow E1*). Likewise, if the key MPs consist of fungicides (*arrow E2*), they may have negative effects on microbial decomposers, thereby potentially reducing breakdown of organic matter. These asymmetric effects may then cascade differentially across the food web. However, the predictions become more complicated given that hundreds to thousands of MPs enter natural ecosystems and due to many trophic interactions (Box 2).

acting in concert with other co-occurring stressors, which may mask or exacerbate pollutant effects (Folt et al., 1999).

(3) *WW generally contains a very large number of different MPs*, which poses two main challenges for predicting the effects of MPs. First, quantifying all MPs present is a scientific challenge due to the immense number of chemicals, thus requiring cutting-edge instrumentation and data-processing tools (Schymanski et al., 2014). Second, the diverse modes of action of these chemicals (see Fig. 2) mean that not all organisms are affected equally and hence only certain parts of the food web may be directly impacted. These effects may subsequently propagate to other organisms via trophic interactions (indirect effects).

Hence, the question how to predict ecological effects due to MPs poses a general ecological problem: How much detail is required—and at which spatial and temporal scales—to enable accurate predictions of how ecosystems respond to (anthropogenic) stressors (see Table 1)? Making scientific progress that allows for accurate ecological predictions in real-world multiple stress scenarios requires not only advances of ecological theory but also sound empirical data (Mouquet et al., 2015). Below, we present the case study *EcoImpact* as one approach to make progress on this empirical aspect.

2. WATER MANAGEMENT AS A *REAL-WORLD EXPERIMENT*

2.1 Making Use of *Real-World Experiments* to Understand MP Impacts

Diverse empirical approaches can be used to tackle the question *How do MPs affect aquatic ecosystems?*, which all have their strengths and weaknesses. On one hand, correlative field surveys allow establishing patterns in nature—but are often accompanied by multiple confounding factors, necessitating a high level of replication to detect general trends, and not allowing direct tests of underlying causal mechanisms (Robinson et al., 2014). On the other hand, highly controlled experiments conducted under simple laboratory conditions may allow inference of the direct impact of specific factors (Benton et al., 2007), but suffer from a lack of ecological realism that makes it difficult to generalize the experimental findings to natural ecosystems (Carpenter, 1996). Experimental manipulations of entire ecosystems (e.g. entire lakes) can be seen as a kind of 'gold standard' and have been pivotal in demonstrating the ecological effects of different external drivers, such as

phosphorus (Schindler et al., 2008), endocrine disruptors (Kidd et al., 2007), or general ecological regime shifts (Carpenter et al., 2011). Such manipulations are, however, rare and generally prevented by economic, logistical or ethical reasons.

Yet, ecosystem manipulations are common for 'nonscientific' reasons—such as management activities aiming to improve the ecological status of water bodies (Bernhardt et al., 2005). This is the basis for what we call *real-world experiments*, which make use of intentionally or unintentionally altered ecological conditions in 'natural' settings, and may allow powerful inferences by combining the benefits of ecologically realistic conditions, experimental manipulations and replication. This approach shares similarities to natural experiments as described by Diamond (1983) but extends this concept explicitly to anthropogenically influenced situations. Of particular relevance is that the monitoring of ecological consequences of specific mitigation measures (e.g. river restoration or WWTP upgrades) can be designed in a (quasi-)experimental manner (as if WWTPs were experimental treatments). Unfortunately, these opportunities have not been systematically capitalized on in the past, as noted for the example of river restoration (Bernhardt et al., 2005).

This is a missed scientific opportunity for applied large-scale ecology. Because ecological mitigation measures are generally implemented (distributed) across large spatial areas and in different ecological contexts, well-designed *real-world experiments* could aid in testing scientific hypotheses about causality and the relative importance of different influencing factors on the ecological status of ecosystems. Furthermore, with such experiments one can systematically evaluate how successfully money has been spent for achieving societal goals by implementing mitigation action (Bernhardt et al., 2005).

The Swiss water sector currently offers a prime opportunity for such real-world experiments. In Switzerland, WWTP infrastructure will be upgraded over the next 20 years to reduce the input of MPs from WWTPs by applying ozonation or powder-activated carbon as an additional treatment step (Box 3). Additionally, many smaller WWTPs will be decommissioned and their WW diverted to larger plants. Following these alterations, water quality in the rivers is expected to change in well-defined ways (e.g. MPs will either be reduced or removed entirely; Fig. 3).

These alterations should result in a measurable biological responses (Fig. 2). With this in mind, we present an interdisciplinary project (*EcoImpact*) that aims to unravel the role of MPs in stream ecosystems. More specifically, *EcoImpact* aims first at establishing the type and magnitude

BOX 3 Upgrading the Swiss WWTP Infrastructure for MP Removal

MPs include an enormous variety of chemical compounds and can enter the environment from various sources (Box 2). To reduce MPs entering the environment, multiple approaches are implemented, reaching from actions at the source (e.g. ban certain compounds) to actions at the end of the WW pipe. One of these measures is upgrading of WWTP infrastructure with an additional treatment step to remove MPs (Abbeglen and Siegrist, 2012). The Swiss government has recently decided that 100 of the 700 Swiss WWTPs will be upgraded during the next 20 years, with the first upgraded plants already being operational. This decision was based on (i) full-scale pilot studies, (ii) social and political acceptance analysis and (iii) technical and economic feasibility assessments (Eggen et al., 2014). Treatment at WWTPs allows the removal of unknown mixtures of MPs to a large extent. With the upgrading, about 50% of Swiss WW will undergo additional treatment.

The Swiss implementation strategy is currently based on two technically feasible and sufficiently cost-effective treatments: ozonation and powder-activated carbon. These two technologies have shown to reduce the total MPs by over 80% (Hollender et al., 2009). As added value of the upgrading, pathogen loads will be reduced by one to three orders of magnitude (Abbeglen and Siegrist, 2012).

At some sites (especially smaller WWTPs), the closure of the plant and transfer of the WW to another, typically larger and neighbouring WWTP is an alternative to upgrading. Locally this should result in an even better water quality at the cost of less discharge in the small streams.

The costs for these additional treatments depend on the condition and size of the WWTP, the technology chosen and the timing of the upgrading. The annual costs for urban drainage and WW treatment in Switzerland are expected to increase by about 6% and its energy consumption between 5% and 30% of its current demand.

(i.e. effect size) of individual to ecosystem-level responses to wastewater-born MPs in streams and environmental contingencies under real-world conditions. Second, it shall elucidate causal relationships between MPs and selected biological endpoints through artificial flume experiments.

In the following sections we will:

- Present a general research strategy where we treat existing WWTPs and their future upgrading as a *real-world experiment* to critically evaluate the ecological relevance of wastewater-born MPs.
- Describe how *EcoImpact* combines highly replicated, interdisciplinary field surveys with manipulative experiments to disentangle the effects of MPs in

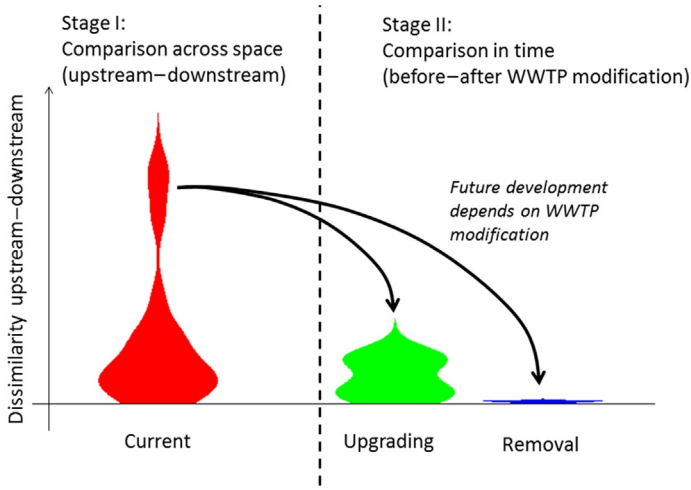

Fig. 3 Conceptual basis for the spatiotemporal design using existing WW treatment plant (WWTP) infrastructure and their planned modifications as *real-world experiments* for testing effects of MPs on aquatic ecosystems. Stage I (US–DS comparison) is based on the spatial comparison between the upstream and downstream locations of the WWTPs (see Fig. 5). The chemical and biological dissimilarities between these locations are quantified (represented by the *red* (*grey* in the print version) *violin plot* for the current conditions). The role of WW and MPs is statistically inferred from the spatially replicated design. Stage II (BACI comparison) compares the dissimilarities before and after the modification of the WWTP infrastructure. Upon upgrading and WW removal, it is hypothesized that the dissimilarities decrease (*green* (*light grey* in the print version) and *blue* (*dark grey* in the print version) *violins*). Because upgrading (only reducing MP load) and removal (reducing MP and nutrient loads) affect water quality differently, this stage allows to disentangle effects of these major WW constituents.

an ecologically relevant context. We show how the study is designed so as to be able to infer general trends (large scale) and context dependency (local scale and interactions among covarying factors, like nutrients) of MP effects (Table 1), and provide first results as proof of principle.

– Provide an outlook for how interdisciplinary, experimental research approaches can address human-induced environmental change in the context of large-scale ecology. We will also consider how inferential power can be increased by combining *real-world* and *research-led experiments*.

2.2 The *EcoImpact* Project as a Case Study

To make use of the opportunity provided by the upgrading of the Swiss WWTP infrastructure, *EcoImpact* was initiated by several departments of Eawag, the Swiss Federal Institute of Aquatic Science and Technology, in

close collaboration with relevant stakeholders (e.g. federal and cantonal authorities, WWTP operators and personnel, etc.). This project combines

(i) replicated *spatial field comparisons* to isolate the effects of WW from other factors of influence (upstream–downstream design),

(ii) replicated *temporal field comparisons* following WWTP upgrading to isolate the effects of MPs from other influence factors (before–after or BACI design; Underwood, 1994). Explicitly, upon upgrading or complete removal of WW, the ecosystems at downstream (DS) sites should become more similar to their upstream (US) reference sites, the extent of convergence depending on the type of treatment (Fig. 3),

(iii) *manipulative experimental approaches* to increase mechanistic understanding and to infer causality, and

(iv) the *expertise of different scientific disciplines* (from engineering and environmental chemistry to ecotoxicology and ecology).

Such a combination of spatial and temporal comparisons offers powerful means for testing the effects of WWTP modifications on relevant ecological endpoints. However, the treatments of this *real-world experiment* are only coarsely defined (e.g. with regard to water quality) and cannot be modified according to scientific demands. Therefore, the field monitoring is complemented by targeted experiments in artificial flumes (described later) to establish causal links between exposure to MPs and the ecological responses.

Given that the environmental driver of interest here is chemical water quality and the expected responses are ecotoxicological and ecological, establishing effects along the effect chain (Fig. 2B) requires the combination of different disciplines and fields of expertise.

2.2.1 Design of the Field Survey

The US–DS comparison of the *EcoImpact* field survey is replicated across 24 sites (Fig. 4). This survey was designed so as to have two US locations as in-stream reference for the impacted DS location at each study site (Fig. 5). The two US reference locations provide a 'null model' for the differences in community composition that could be explained by distance alone (within a given stream) (Fig. 3; Burdon et al., 2016). In the BACI part of the study (Stage II), these US sites will provide the 'null model' for the temporal changes that may occur irrespective of the WWTP modifications. In combination, this design enables us to test both general responses to wastewater discharge and the environmental contingency of the effects.

In the field survey, well-established chemical, ecotoxicological and ecological methods are implemented (Table 3; Box 5). In doing so, these data

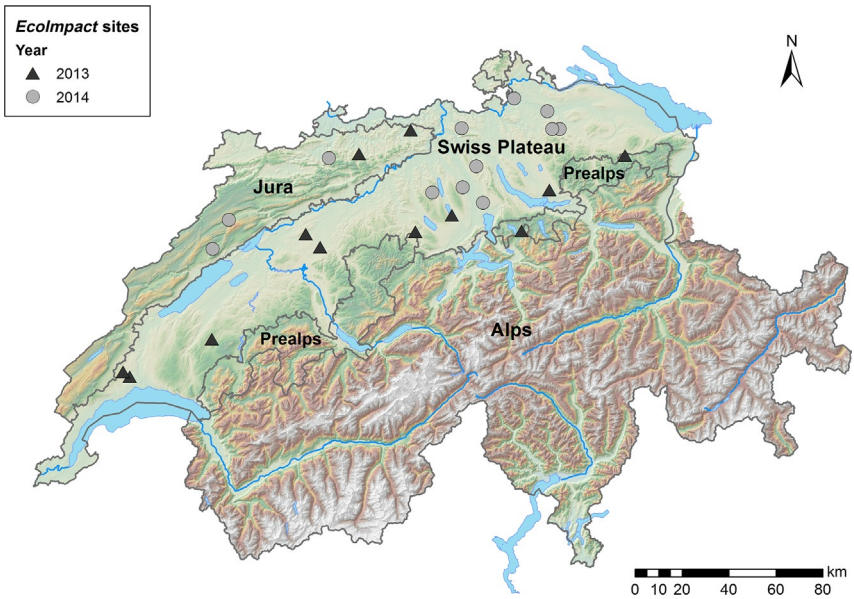

Fig. 4 Map of the 24 *EcoImpact* field sites distributed across three biogeographical regions Jura, Swiss Plateau, and Pre-Alps. *Swisstopo: 5704 000 000/DHM25@2003; Vector200@2015; swissTLM3D@2014, reproduced with permission of swisstopo/JA100119.*

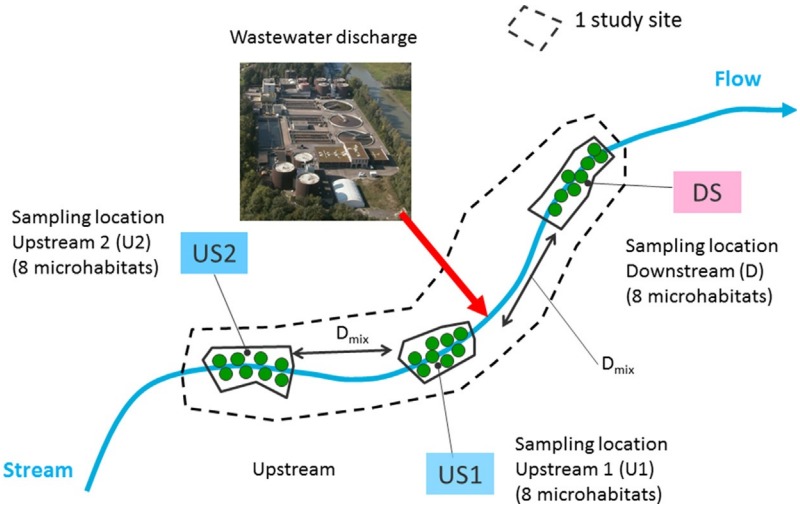

Fig. 5 Schematic of the layout of the sampling design for the US–DS comparisons. In each of the 24 streams, a study site consists of one DS location (*DS*), where the WW is fully mixed across the river cross-section, and two US locations (*US1*, *US2*). The US locations are placed at equidistant distances upstream of the WWTP and control for the effect of geographic distance and thereby allow for a comparison with the downstream location (DS) as well as for an in-stream control (US2–US1). Further details are given in the text and in Burdon et al. (2016). *Picture WWTP: Copyright/Author: Christoph Lüthi, Sandec and Eawag.*

Table 2 Quantitative Criteria Used for the Selection of Sites

Category	Site Property	Description	Threshold
Wastewater	Effluent upstream	Proportion of discharge (at Q_{347})	0%
	Effluent downstream	Proportion of discharge (at Q_{347})	>20%
Land use	Urban areas	Areal fraction of watershed	<21%
	Special crops (e.g. orchards, vegetables)	Areal fraction of watershed	<10%

Q_{347}: Discharge that is statistically exceeded at 347 days per year (95% low-flow conditions).

can also be put in context with landscape-level processes driving local and regional biodiversity (Altermatt et al., 2013; Burdon et al., 2016).

The 24 sites are located in the Swiss Plateau, Jura Mountains and Pre-Alps regions of Switzerland (Fig. 4). The high site replication is essential to account for effects across a wide range of environmental contexts (e.g. different catchment characteristics) upstream of the WWTPs, including different biogeographical regions. The sites were chosen so that the differences in the fraction of WW between US and DS locations of the selected WWTPs were as large as possible (Burdon et al., 2016). These requirements were realized by (i) applying specific criteria relating to land use, discharge and regional coverage during site selection (Table 2) and (ii) ensuring that conditions between US and DS sampling locations within a given location were as similar as possible with regard to surrounding land use, stream morphology, and riparian vegetation. Other confounding factors between sampling locations (e.g. confluence with a tributary) were similarly avoided. Because of these criteria, all sites consist of lower-order streams (because larger rivers invariably receive WW above potential WWTPs). For logistic reasons, the US-DS (Stage I) field study was conducted over 2 years (12 sites in 2013 and 12 sites in 2014).

2.2.1.1 Quantifying Environmental Drivers

To quantify pollutant exposure as well as putative confounding/correlated factors of water quality, water samples were regularly (mostly monthly/bi-monthly) collected for the determination of 20 basic water chemistry parameters (e.g. major ions, concentrations of MPs and heavy metals during base flow conditions, analytical method see Box 5). Benthic habitat characteristics were also recorded to account for context dependency (methods see

Box 5). These measurements enable testing of the association of physicochemical environmental parameters with biological endpoints.

2.2.1.2 Biological Endpoints

A variety of biological responses relevant for assessing ecological effects of pollution were measured across four levels of biological organization from individuals, to populations, communities to ecosystem functions (Fig. 2). For instance, samples for in vitro ecotoxicological assays were taken once each year (May/June) simultaneously with the regular water samples. These assays covered endpoints that are relevant for different trophic levels: algae (inhibition of photosynthesis (Escher et al., 2008)), invertebrates (inhibition of acetylcholinesterase (Hamers et al., 2000)) and vertebrates (endocrine disruptors; YES-test (Routledge and Sumpter, 1996)). The ecological endpoints ranged from microbial and macroinvertebrate community descriptors (Burdon et al., 2016), and age structure of *Gammarus* amphipods, to leaf litter decomposition and microbial-mediated cotton strip breakdown rates (see Table 3).

2.2.1.3 First Insights

The design of the field study outlined before resulted in first outcomes for the Stage I of the project, which is the spatial US–DS comparison. The BACI design of Stage II can only be realized once a number of WWTPs have actually undergone upgrading.

The selection of the field sites indeed resulted in a broad range of ecological conditions upstream of the WWTPs. For example, catchment areas covered by forest and grassland ranged from 10% to 80% and arable land varied between 0% and 50% (see also Burdon et al., 2016). Correspondingly, also water quality varied strongly. We illustrate this aspect with variation in nitrate concentrations at the US locations (Fig. 6): the more the catchment was dominated by grassland and forests (i.e. the less arable land), the lower the nitrate concentrations. Importantly, these US landscape conditions correlated with the composition of the macroinvertebrate community at the US sampling locations: The larger the fraction of grassland or forest—land use types without substantial use of pesticides—the larger the observed fraction of species considered sensitive towards pesticides as indicated by the SPEAR Index (Fig. 6; see also Burdon et al., 2016).

This observed variation across the 24 sites offers the possibility to study how ecological effects caused by discharge of WW may depend on the ecological context (Burdon et al., 2016). To answer this question, one has to

Table 3 Key Ecological Responses Measured in the US–DS Field Surveys and Three Flume Experiments in *EcoImpact*

Biological Level	Response	Method and/or Organism(s) Used	Description	References for Methods	Field	Flumes
Individuals/ population	Secondary production	*Ancylus*, *Gammarus*	Individual growth (% change in body length)	Hicklen et al. (2006), Watts et al. (2002)	No	Yes
	Fitness	*Ancylus*, *Gammarus*	Survival and condition of invertebrates (condition factor length:mass ratio)	Gerhardt (2011), Rosés et al. (1999)	No	Yes
	Demography	*Baetis*, *Gammarus*	Hess sampling, size distribution and population density	Ladewig et al. (2005)	Yes	No
Community	Detrital consumption	Leaf discs, *Gammarus*	Leaf disc consumption	Mancinelli (2012), O'Neal et al. (2002)	No	Yes
	Biofilm consumption	Plastic slides, *Ancylus*	AFDM of biofilm consumed per day	Rosemond et al. (2000)	No	Yes
	Invertebrate community composition	Semiquantitative sampling	Handnet used to collect invertebrates from standardized area and locations in channels, different diversity measures (e.g. EPT taxa, SPEAR Index, Saprobic Index)	Stucki (2010)	Yes	Dilution experiment only
	Algal (diatom) community composition	Semiquantitative sampling	Sample collected from stones, tiles and glass slides before preservation and identification	Biggs and Kilroy (2000)	Yes	Spiking experiment only

Continued

Table 3 Key Ecological Responses Measured in the US–DS Field Surveys and Three Flume Experiments in *EcoImpact*—cont'd

Biological Level	Response	Method and/or Organism(s) Used	Description	References for Methods	Field	Flumes
Ecosystem	Biofilm production	Tiles	AFDM of standing biomass, primary producers (chlorophyll *a*), resource quality (C:P, C:N ratios)	Biggs and Kilroy (2000), Lamberti and Resh (1985)	Yes	Yes
	Detrital processing	Leaf packs (coarse and fine)	AFDM loss per day measured for standard litter source (Black Alder (*Alnus glutinosa*) leaves)	Bärlocher (2005), Benfield (2007)	Yes	Yes
	Decomposition	Cotton strips (standardized carbon source cellulose)	Respiration (microbial activity), mass loss and tensile strength loss	Slocum et al. (2009), Tiegs et al. (2013)	Yes	Yes
	Ecosystem metabolism	Single-station oxygen logging	Change of oxygen concentrations as ecosystem response	Bott (1996), Stephens and Jennings (1976)	Yes	Yes

The chosen response variables are standard parameters used in ecological studies and reflect different parameters from the individual to ecosystem levels. Study organisms were chosen so as to represent taxa that are sensitive to water quality (e.g. diatom or macroinvertebrate communities) and/or play different key roles in stream ecosystems (e.g. periphyton and *Gammarus*–leaf litter interaction). *AFDM*, ash-free dry mass. See Box 4 for details on flume experiments.

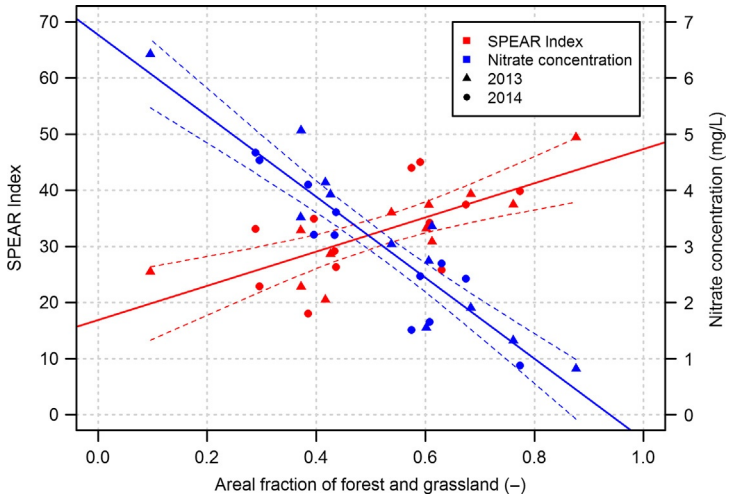

Fig. 6 Correlations between upstream land use (*x*-axis) and (A) the nitrate concentrations and (B) the SPEAR Index (a measure how many taxa sensitive against pesticides are present in a macroinvertebrate community (Liess and von der Ohe, 2005)) at the upstream locations of the 24 *EcoImpact* sites with 95% confidence intervals for the expected values (*dashed lines*). *Triangles* depict the 2013 sites, *circles* the 2014 sites. Details of the methods are provided in Burdon et al. (2016) and in Box 5.

consider to which extent water quality differs between the US and the respective DS location. The impact of WW discharge on DS water quality depends on the composition of the discharged WW, its fraction to total flow in the stream and on the US water quality. As expected, concentrations of chemicals (e.g. nutrients and MPs) as well as the microbial load increased in the DS section at all 24 *EcoImpact* sites (Fig. 7). The magnitude of the changes however differs among specific WW components and is particularly striking for some MPs (such as pharmaceuticals) which increased up to 30-fold (Fig. 7). Despite this general increase downstream, a closer look reveals that WW composition with regard to MPs and nutrients varied considerably between sites (Fig. 8).

The macroinvertebrate communities at DS locations clearly responded to WW input. They were characterized by reduced abundance of sensitive taxa such as the EPT (Ephemeroptera, Plecoptera, Trichoptera) fauna (Burdon et al., 2016). On the other hand, DS sites showed a pronounced increase in oligochaete worms (Burdon et al., 2016), a general pattern seen in classical WW studies (e.g. Hynes, 1963), which seems to persist despite the advances in (conventional) WW treatment over the last decades.

The context dependency, however, varied between the metrics for characterizing the macroinvertebrate communities. For example, the more

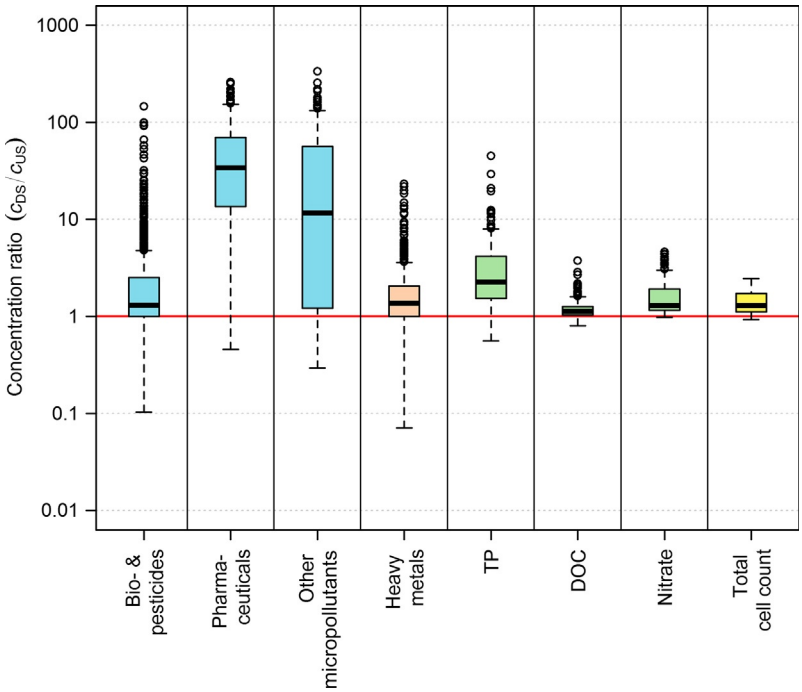

Fig. 7 *Boxplots* depicting the ratios between downstream (*DS*) concentrations and upstream concentrations (*US*) for different water quality parameters at the 24 *EcoImpact* sites across all sampling dates. The *red line* (*grey* in the print version) (ratio = 1) indicates that there is no difference between DS and US sites within a given stream. Values >1 indicate that the concentration of a given parameter is higher DS than US. *Bio- and pesticides*, concentration sum of all measured biocides and pesticides (31 compounds); *cell counts*, concentration of bacterial cells; *DOC*, dissolved organic carbon; *heavy metals*, concentration sums of the 10 most abundant heavy metals; *other MPs*, concentration sums of 5 compounds; *pharmaceuticals*, concentration sums of 21 compounds; *TP*, total phosphorus. Box size corresponds to the first and third quartiles (25% and 75% quartiles); whiskers extend to the most extreme data point which is no more than 1.5 times the length of the box away from the box. Details of the methods are provided in Box 5.

pristine the US conditions, the larger the change in the Saprobic Index at DS locations (Burdon et al., 2016). This variation in community-level responses is associated with background nutrient levels and, hence, the agricultural land use US of the WWTPs. In contrast, a larger fraction of WW leads to a more pronounced loss of sensitive macroinvertebrates at DS locations (i.e. SPEAR Index), irrespective of US conditions, indicating that some changes caused by discharged WW may not be environmentally contingent.

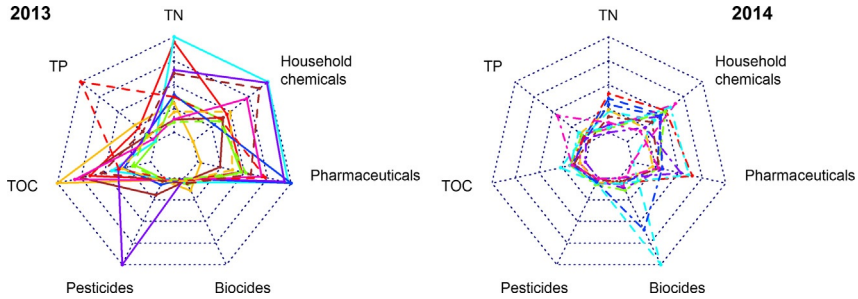

Fig. 8 *Spider diagrams* depicting the WW composition at the 24 *EcoImpact* sites. The *left* figure shows the results for the 2013 sites (one *colour* (*grey* in the print version) per site), the *right* for the 2014 sites. For each axis, the average concentrations of the respective January and June samples per site are normalized to the maximum value of all sites. *TOC*, total organic carbon; *TN*, total nitrogen; *TP*, total phosphorus; household chemicals (5 MPs); pharmaceuticals (21 MPs); biocides (3 MPs); pesticides (28 MPs).

Future analyses on the other biological endpoints (Table 3) will yield additional insights how effects of (specific) MPs may depend on the ecological context (ongoing work).

2.2.2 Inferring Causality (Flume Experiments)

Despite the merits of the *real-world experiment* approach described earlier, its potential for gaining insights into causal links between MPs and ecological effects is enhanced in combination with *research-led experiments*. To that end, we developed an artificial stream system (*Maiandros*) where contaminant concentrations can be manipulated through either dilution or spiking experiments (Box 4). The system is placed directly at a WWTP, which provides direct access to treated WW (allowing dilution experiments) and to WW treatment (allowing disposal of pollutants in spiking experiments).

The development of such experiments requires a substantial simplification compared to the natural system and is not a trivial task. This holds true both for water quality and for the biological endpoints under study. First, as there are thousands of MPs in streams receiving WW discharge (Box 2), how does one choose which ones to include? Second, as the impacts of MPs with different modes of action (Fig. 2) as well as their expected ecological relevance strongly depend on the study organism, which ecological endpoints are relevant to study? These quandaries touch upon the fundamental questions of how holistic experiments in ecology can actually be, and if ecology is inherently a reductionist science (Bergandi and Blandin, 1998). Here,

BOX 4 Experimental Flume System: *Maiandros*

Artificial flume systems allow experimental tests of causality of different water constituents on stream ecosystems and have been implemented by different institutions (Bruder et al., 2015; Grantham et al., 2012). Following such ideas, the *EcoImpact* team designed the flume system *Maiandros* that allows experimental manipulation of WW and replicated randomized experiments. *Maiandros* currently consists of 16 parallel channels (Fig. B.2) for running surface water experiments. The system allows for quantitatively mixing two different influents (e.g. local surface water with biologically treated WWTP effluent at different dilutions, or spiking experiments with known concentrations of MPs). Four mixing units are available; the blended influent of one water quality is distributed evenly to four flow channels. Each channel is doughnut shaped (length: 4 m, width: 0.15 m, water depth: 0.1 m) and is equipped with a paddle wheel. The typical hydraulic retention time is about 7 min.

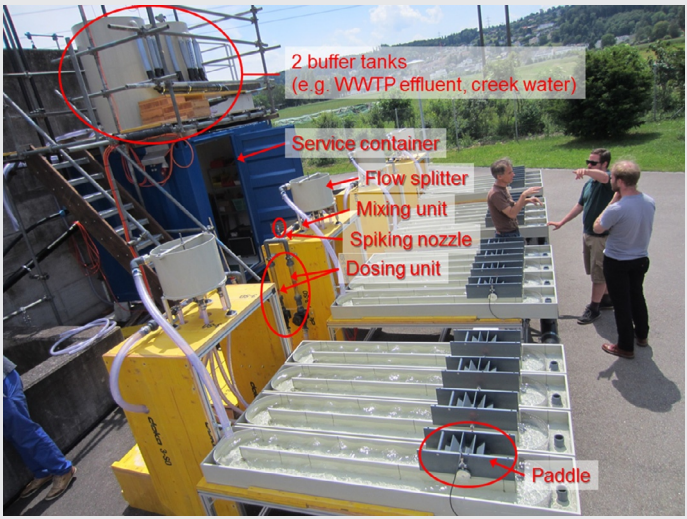

Fig. B.2 The *Maiandros* flume system consisting of 16 flumes arranged in four blocks before launching the first experiment. Each of the four treatment combinations (e.g. four levels of dilutions or different combinations of MP and nutrients) is present in each block; within the blocks, the treatments are randomly assigned to the flumes. *Picture/copyright: A. Joss, Eawag.*

The design and implementation of such a flume system for MP studies in an ecologically relevant context is a technological challenge that requires expertise across all disciplines. Aspects to be addressed range from technical (e.g. how to keep mixtures of compounds in solution and which materials have the right mechanical and chemical properties) to ecological aspects (e.g. appropriate flow velocities, which organisms are suitable for such experiments and how to establish them in the flumes). Hence, as for the conceptual aspects and methodological of the project as a whole, interdisciplinarity is a key factor for a successful implementation.

EcoImpact takes a reductionist view in that we try to reproduce key findings from the field by testing causal hypotheses of potential drivers of change.

To that end, we followed a stepwise procedure. First, dilution experiments maintain the full chemical complexity of WW by exposing selected organisms to a range of mixtures between river water and treated WW (20%, 50%, 80%) and compare the biological effects to a control consisting of river water only (Exp. I). The selected biological endpoints included periphyton biomass and community tolerance (PICT assays; Tlili et al., 2015), performance (growth and survival) of freshwater crustaceans (gammarid amphipods) and molluscs (ancyclid snails) and ecosystem functions (see Table 3). Because several of these endpoints (e.g. cotton strip degradation, PICT assays; Table 3) were also measured at the field sites, this experiment allows a comparison of effects in situ in the field and in the flumes.

Second, spiking experiments substitute real WW by artificial mixture of chemicals and thereby allows disentangling the effects of MPs and nutrients (the main correlative factor in WW; Exp. II and Exp. III). In our case, we chose a mixture of 17 compounds (see Box 5) that are representative of MPs from our field sites and, hence, shared sufficient similarity with real-world mixtures to be of ecological relevance. In particular, frequently detected compounds with different modes of action (e.g. pesticides or pharmaceuticals) were selected. Exp. II, consisted of fully factorial combination of MP (present or not) and nutrient (present or not) treatments, compared to a control treatment (100% stream water). In Exp. III, the 'nutrients only' treatment was replaced by a technical control, to account for the possible effects of methanol used as solvent for the MPs. In all of these experiments, we applied a constant dilution or concentration treatment during 4 weeks.

In the field, such a disentangling of MP and nutrient effects will only be possible during Stage II (Fig. 3) once there is a sufficient number of WWTPs that have either been upgraded or shut down. In the flumes, this time lag can be avoided and we have obtained first results disentangling effects of MPs and nutrients (Exp. II and Exp. III). Results from Exp. II demonstrate that diatom communities that established on glass slides (method description see Box 5) in river water spiked with MPs have a reduced average biovolume as compared to the controls (Fig. 9) (Reyes, 2015). Spiking only nutrients to the river water, however, caused larger biovolumes. These findings suggest that MPs exert effects on ecologically important groups such as diatoms that

BOX 5 Methods Used in *EcoImpact*

The results briefly presented in our contribution, range from data on water chemistry and macroinvertebrate community responses in the field surveys to diatom community responses in Exp. II in the flumes (MP spiking experiment).

Water chemistry: Water chemistry was characterized both in terms of general composition (e.g. hardness, nutrients and major ions) and in terms of different types of pollutants (e.g. heavy metals, organic MPs, and oestrogens). General water chemistry, including nutrient levels, was quantified using standard methods of the Swiss National River Monitoring and Survey Programme (NADUF; http://www.bafu.admin.ch/wasser/13462/14737/15108/15109).

For the analysis of heavy metals, samples were acidified (0.6% HNO_3) and quantified with high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS; Element2, Thermo, Switzerland). Details can be found in Meylan et al. (2003). Here, we report the data of unfiltered samples (Figs 7 and B.1).

For the analysis of organic MPs (see Figs 7, 8 and B.1), the samples of the June 2013 campaign were enriched with offline solid-phase extraction (SPE) as described in Kern et al. (2009) using manually packed mixed-mode cartridges (Schollee et al., 2015), whereas all the other samples were enriched with an automated online SPE similar to Huntscha et al. (2012). Liquid chromatography high-resolution mass spectrometry (LC-HRMS) and the subsequent quantification were performed as described in Schollee et al. (2015) and Huntscha et al. (2012).

Oestrogen levels (Fig. B.1) were determined with the Yeast Oestrogen Screen test ('YES Test'; Routledge and Sumpter, 1996). This method relies on genetically modified yeast cells (*Saccharomyces cerevisiae*) containing the gene for the human oestrogen receptor coupled with a 'reporter gene' (LacZ). Oestrogenically active substances bind to the oestrogen receptor in the cell activating the reporter gene. This gene encodes for an enzyme (beta-galactosidase), which converts a dye from yellow to red. This process allows for photometric determination of the concentration of oestrogenically active substances.

Macroinvertebrate survey: The invertebrate sampling methods followed standard protocols for benthic macroinvertebrate biomonitoring in Switzerland (Stucki, 2010). Details for the methods can be found in Burdon et al. (2016) which shows responses for sites sampled in 2013. The presented results (Fig. 6) combine both 2013 and 2014 data. Briefly, at each sampling location (DS, US1, US2) in Spring 2013 and 2014 benthic invertebrates were sampled using pooled kick nets (25×25 cm opening, 500-μm mesh size) with a standardized sampling effort covering all major microhabitats found within each reach. Samples were stored in 80% ethanol prior to identification to the family level using relevant literature (Stucki, 2010). Macroinvertebrate communities were described using diversity-based indices, and two trait-based indices (Saprobic Index and SPEAR Pesticides Index) (Burdon et al., 2016).

BOX 5 Methods Used in *EcoImpact*—cont'd

The Saprobic Index ranges between 1 and 4 and increases with greater fractions of taxa that are tolerant to low oxygen conditions (Bunzel et al., 2013). Following the methods outlined in Burdon et al., 2016, the Saprobic Index was calculated using German saprobic trait values obtained from: http://www.freshwaterecology.info. This online resource is a taxa and autecology database for freshwater organisms (Version 5.0, Date accessed: 26.03.15; for more information, see Schmidt-Kloiber and Hering, 2015). The SPEAR Pesticides Index (SPEAR Index) describes the percentage of taxa susceptible to pesticides and was calculated using the SPEAR Calculator (Version 0.9.0, downloaded 17.2.16). Lower relative abundances of SPEAR taxa indicate pesticide stress and it is used extensively as an index of stream health in Europe (Beketov et al., 2009; von der Ohe and Liess, 2004).

Spiking experiments: Results from two spiking experiment in the *Maiandros* flumes (Box 4) are mentioned in the text (Exps II and III). In these two experiments, the MP mixture consisted of the following compounds (with nominal spiking concentration (ng L^{-1}) in the flumes given in parentheses): amisulpride (106), atenolol (217), benzotriazole (1098), candesartan (722, Exp. II only), carbamazepine (283), citalopram (55), clarithromycin (63), diazinon (4570 Exp. II, 457 Exp. III), diclofenac (603), diuron (75), β-estradiol (0.35), fexofenadine (322), tebuconazole (24), iopromide (1629), metformin (5014), sucralose (1175), triclosan (55), valsartan (677, Exp. III only) and Zn (6040). The increase of nutrient concentrations in the flumes due to spiking amounted to 0.05 P, 0.076 NH_4-N and 1.55 nitrate-N (values in mg L^{-1}).

Diatom data: Diatoms are sensitive bioindicators and readily respond to pollution (Visco et al., 2015). In *Maiandros* Exp. II, three glass slides (75 × 50 × 1.1 mm) were placed in each of the 16 channels, completely submerged and oriented in flow direction (16 October 2014). Prior to the experimental treatments, the slides were conditioned in the flumes with flowing river water for 1 week, so that all treatments would have a similar initial biofilm community. Subsequently, the glass slides (i.e. the biofilm community) was exposed to a combination of MPs and nutrients for 4 weeks (from 23 October to 11 November 2014). Subsequently, the biofilms were scratched off from both sides of the slides with a spatula into a centrifuge tube and the biofilm was immediately fixed with formaldehyde (4%). (Details can be found in Reyes, 2015.) One sample per channel (i.e. four replicates per treatment) was randomly selected and prepared for morphological identification of diatoms according to Hürlimann and Niederhauser (2007). Briefly, samples were first homogenized, subsampled and freed from coarse matter with hydrochloric acid. Subsequently, the samples were oxidized with sulphuric acid and potassium nitrate in order to remove all organic matter, leaving only the silica shells of the diatoms for later identification and measuring of cells (i.e. biovolume). Finally, the diatom valves were embedded with Naphrax into a glass

Continued

BOX 5 Methods Used in *EcoImpact*—cont'd

slide for microscopy. A total of 500 diatom valves per sample were identified to species level (when possible) or genus level after (Hofmann et al., 2013) with an inverted Leica Microscope and a x1000 magnification.

In order to characterize the biovolume of the community the dataset from Rimet and Bouchez (2012) was used. The volume for each species was calculated as *Length* × *Width* × *Height*. Size was corrected for all species accounting for at least 10% of abundance in each treatment with the mean value of 10 valves.

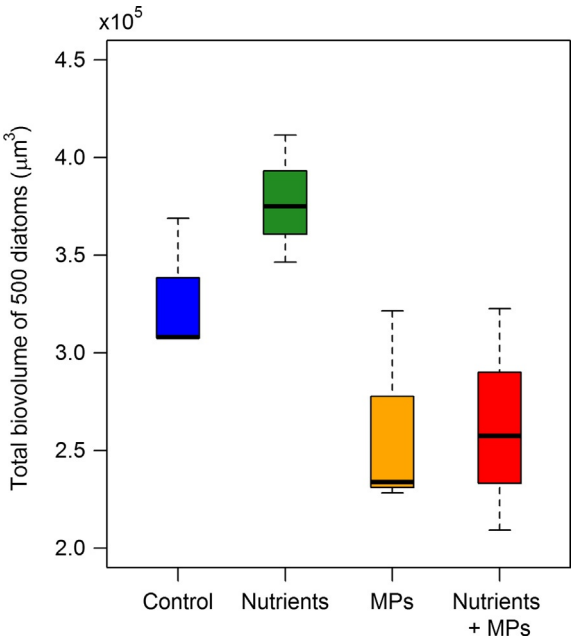

Fig. 9 Comparison of diatom biovolumes in a MP spiking experiment (Exp. II). In this experiment, glass slides were placed in the *Maiandros* flumes and diatoms allowed to establish under the four water treatments during 4 weeks (fall 2014). The treatments consisted of a control (C = River water only); MPs only (MP), MP + with nutrients (nitrogen N, phosphorus, P) and nutrients only (N, P). The MP treatment consisted of spiking with a known concentration of mix of 17 MPs (see Box 5). Box sizes correspond to the first and third quartiles (25% and 75% quartiles); whiskers extend to the most extreme data point which is no more than 1.5 times the length of the box away from the box. *Data from Reyes, M., 2015. Auswirkungen von Mikroverunreinigungen und Nährstoffen von Kläranlagen auf Populationsstrukturen von Kieselalgen. Zertifikatsarbeit CAS Zürcher Hochschule für angewandte Wissenschaften, Wädenswil.*

override subsidy effects of nutrients. Upcoming work will analyse this aspect in more depth (including results from Exp. I to Exp. III).

3. OUTLOOK: POTENTIAL OF COMBINING *REAL-WORLD* AND *RESEARCH-LED EXPERIMENTS*

3.1 Planned Changes in Urban Infrastructure as *Real-World Experiments*

The current comparison of US to DS locations at 24 *EcoImpact* sites allows inferences on how WW may affect stream ecosystems—from physiological responses to ecosystem functions (Fig. 2; Table 3). In the future, upgrading of the WWTPs (Box 3) will provide an explicit opportunity to study the effects of altered water quality (i.e. removal of MPs or composite WW) on ecosystems across a large spatial range over time (Fig. 3). Due to the in-stream controls (Fig. 5), such temporal changes should be detectable against simultaneously occurring intrinsic changes that are independent of the changes related to WW (i.e. temporal changes that also occur in the US sections).

As promising as this approach looks, it comes with challenges that are of general relevance and can be exemplified with *EcoImpact*. First, to provide mechanistic insight, it is necessary to monitor key variables of interest in the long term both because not all responses may be immediate and to test how the initial effects change over time. Hence, one major issue to be solved is the long-term establishment of systematic observations. But also human interventions, such as implemented mitigation measures, used for the *real-world experiment* are not all realized simultaneously. For instance, it will take years for implementing all of foreseen WWTP modifications. Hence, Stage II of *EcoImpact* (BACI design) will last many years, a time span that goes beyond those of typical research projects. Due to financial and methodological constraints, monitoring programs of public authorities may not fully cover such needs. We emphasize that research institutions may benefit from contributing to such long-term observation given the opportunity this provides for gaining relevant long-term data (Lindenmayer et al., 2012). This in turn would allow, for example, studies on the interactions between ecological and evolutionary processes (Hairston et al., 2005; Moya-Laraño et al., 2014; Travis et al., 2014) of natural systems due to altered pollution patterns. Additional potential for long-term observations could be realized through the contributions of 'citizen scientists' (Bonney et al., 2009; Huddart et al., 2016). The usefulness of this approach has been recently demonstrated in following the ecological consequences of a pesticide spill in the United Kingdom (Thompson et al., 2015).

However, for really large-scale efforts, citizen science would likely not be sufficient. Here large-scale collaborations using highly standardized approaches, such as in the Nutrient Network (a globally distributed experimental network testing the effects of nutrient additions and food-web manipulations across terrestrial biomes) (Borer et al., 2014), may provide powerful means for answering big questions at a large scale. Given that WWTPs are common features of urban infrastructure in many countries around the globe and WW upgrades are conducted in several developed countries, such studies would allow detecting general trends and context dependency of MP impacts at large scales, as well as increase our understanding of ecosystem responses to environmental change in general.

3.2 *Real-World* and *Research-Led Experiments* in Large-Scale Ecology

We started off with the observation that environmental scientists often face a two-fold challenge in providing reliable answers at large scales to inform policy change on one hand, and local scale management advice on the other. Typically, such problems consist of multiple stressor situations. Important questions are then: how to identify the drivers of change at large and local scales, and what are the general (large-scale) trends in the ecological responses to the drivers like nutrient dependencies of litter decomposition at continental scale (Woodward et al., 2012), and when does local context dominate?

Above, we presented an interdisciplinary research approach to tackle such questions in the context of WW-borne MPs. We propose that the combination of *real-world experiments*, which explicitly take advantage of the implementation of mitigation measures in practice, and *research-led experiments* (as characterized in Table 4) provides a powerful tool for detecting general patterns (trends), elucidating the role of context dependency (e.g. local deviations from such trends) and establishing causality between external drivers and the biological responses. Such an approach broadens the scope for gathering relevant ecological data at large temporal and spatial scales and can be generalized beyond the problem of the ecological effects MPs as a tool for increasing inferential power of studies on ecosystems responses to human impact.

The major advantages of combining these approaches can be described as follows:

1. *Benefit from real-world experiments*. Although human interventions often result in multiple changes simultaneously, there are situations where a

Table 4 Comparison of *Real-World* and *Research-Led Experiments*

	Real-World Experiments	***Researcher-Led Experiments***
Advantages	• Response observed under full complexity • One to many replicates • Broad range of ecological context • Allows for detection of real-world trends • Facilitates communication with practitioners and society	• Designed according to scientific interests • Targeted at understanding mechanisms and establishing causality • Interpretation improved due to reduced complexity • Correlated influence factors can be separated • Randomized designs
Disadvantages	• Not designed to address scientific research questions • Possible mismatch between practical and scientific interests • Treatment categories may be coarse • Limited possibility for elucidating mechanisms and establishing causality • Several influencing factors may be correlated	• Artificial (boundary) conditions may limit transferability to and relevance for real-world situations
Layout	• Selection from existing situations (see Table 2)	• Predesigned experiment

Some exceptional experiments like whole-lake manipulations (Kidd et al., 2007; Schindler et al., 2008) may share features of both categories.

single factor (i.e. main driver) differs between points in space or time. By carefully identifying such situations, it is possible to layout a (quasi-) experimental design that allows both to isolate the influence of a single factor and to obtain generality at large scales due to the observation a broad range of ecological context

Key features of such a design are:

(i) Avoiding confounding factors (for example, changes of stream morphology between US and DS sites that are not due to WW input) by careful choice of study sites.

(ii) Quantifying the drivers of change—as well as putative correlated and confounding factors—to the best possible degree (e.g. composition of WW to test whether specific ecological effects are caused by specific MPs).

(iii) Including proper controls to account for confounding factors that may cause temporal changes irrespective of the treatments (e.g. altered ecological conditions due to climate change or the appearance of nonnative species).

(iv) Replicating in space and time. Only replication will allow to establish generality of findings, to investigate context-dependencies and to minimize the risk to overinterpret idiosyncratic, site-specific results (Nakagawa and Parker, 2015).

2. *Combine real-world experiments with research-led experiments*. While the appropriate experimental design in any *real-world experiment* is mainly achieved by a careful selection of existing situations, complementary classical *research-led experiments* are designed by scientists on purpose to test specific hypotheses. A key advantage of such integration is the possibility to separate different influence factors that are highly correlated in the real world, by replicated and randomized experiments. To make such experiments complementary to the *real-world experiments*, they should be designed as plausible simplifications of the real-world situation but still share common biological endpoints. Due to the inherent complexity of ecosystems, different types of manipulative experiments (lab, mesocosm, and field experiments), as well as measurements of different biological endpoints at different hierarchical levels across the ecosystem are needed (see Fig. 2).

3. *Benefit from a broad interdisciplinary team*. When the pertinent questions at hand—typical for ecologically relevant questions—reflect different interacting processes (such as chemical water quality, resulting toxicity and different biological responses), it is necessary to apply different methods and to collect and analyse vastly different types of data. Hence, collaborations across different research fields are mandatory. The common framing of the specific scientific question(s) and of an explicit conceptual framework for linking the results from the different disciplines is essential.

4. *Benefits of combining hypothesis-driven and exploratory approaches*. As ecosystems are inherently complex systems, any framing of the scientific question(s) and the underlying hypotheses is contingent on the current level of understanding. From a heuristic perspective, combining hypothesis-driven and exploratory approaches that widen the scientific view beyond the initial research question enhances the chance to identify blind spots in the current knowledge.

Real-world experiments have substantial additional advantages that make them interesting in the context of large-scale ecology. The 'implementation' of

these experiments (not the scientific analysis, of course) comes for free to researchers and can be considered a 'legal' intervention in the environment. The significance of this argument becomes obvious when contrasted with the scenario of designing a purely scientific experiment to manipulate water quality at such large scales in real systems for studying ecological responses. Although there are well-known manipulative experiments on streams and lakes which were essential for advancing our ecological understanding (Kidd et al., 2007; Schindler et al., 2008; Woker and Wuhrmann, 1957), such experiments are generally too costly and contradict legal requirements regarding water protection and may raise serious ethical issues. Hence, if such experiments are going to happen for other reasons, science should take the opportunity to benefit as well. Given the large number of interventions into aquatic ecosystems, using them as *real-world experiments* can also be considered as one way that turns ecology into a branch of 'big data' science (Hampton et al., 2013).

Finally, such *real-world experiments* facilitate—according to our experiences—the interactions between researchers and stakeholders. When communicating with practitioners, it is essential to demonstrate the impact of scientific findings for practical situations that stakeholders are familiar. This transfer process is often not easy to establish. When working in the context of *real-world experiments*, this obstacle largely vanishes because the research results are obtained from situations with which both researchers and practitioners are familiar with. This aspect may get strengthened further if (long-term) observational studies are carried out in collaboration with citizen scientists and practitioners.

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