Effects of Anthropogenic Land Use and Associated Stressors on Stream Ecosystems

by

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Declaration

I hereby declare that I autonomously conducted the work presented in this Ph.D thesis entitled "Effects of Anthropogenic Land Use and Associated Stressors on Stream Ecosystems". All used assistances and involved contributors are clearly declared. This thesis has never been submitted elsewhere for an exam, as thesis or for evaluation in a similar context; to any department of this university or any scientific institute. I am aware that a violation of the aforementioned conditions can have legal consequences.

Landau in der Pfalz,

Place, date Signature

Overview of Publications

This cumulative dissertation is adapted from the following three scientific publications:

- Peters K, Bundschuh M, Schäfer RB (2013) Review on the effects of toxicants on freshwater ecosystem functions. *Environmental Pollution* 180: 324 – 329.
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"You cannot get through a single day
without having an impact on the world around you.
What you do makes the difference,
and you have to decide
what kind of difference you want to make"
- Jane Goodall -

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[1]*Dendemann* - [2]*Marteria*

SUMMARY

World's ecosystems are under great pressure satisfying anthropogenic demands, with freshwaters being of central importance. The Millennium Ecosystem Assessment has identified anthropogenic land use and associated stressors as main drivers in jeopardizing stream ecosystem functions and the biodiversity supported by freshwaters. Adverse effects on the biodiversity of freshwater organisms, such as macroinvertebrates, may propagate to fundamental ecosystem functions, such as organic matter breakdown (OMB) with potentially severe consequences for ecosystem services. In order to adequately protect and preserve freshwater ecosystems, investigations regarding potential and observed as well as direct and indirect effects of anthropogenic land use and associated stressors (e.g. nutrients, pesticides or heavy metals) on ecosystem functioning and stream biodiversity are needed. While greater species diversity most likely benefits ecosystem functions, the direction and magnitude of changes in ecosystem functioning depends primarily on species functional traits. In this context, the functional diversity of stream organisms has been suggested to be a more suitable predictor of changes in ecosystem functions than taxonomic diversity.

The thesis aims at investigating effects of anthropogenic land use on (i) three ecosystem functions by anthropogenic toxicants to identify effect thresholds (chapter 2), (ii) the organic matter breakdown by three land use categories to identify effects on the functional level (chapter 3) and (iii) on the stream community along an established land-use gradient to identify effects on the community level.

In chapter 2, I reviewed the literature regarding pesticide and heavy metal effects on OMB, primary production and community respiration. From each reviewed study that met inclusion criteria, the toxicant concentration resulting in a reduction of at least 20% in an ecosystem function was standardized based on laboratory toxicity data. Effect thresholds were based on the relationship between ecosystem functions and standardized concentration-effect relationships. The analysis revealed that more than one third of pesticide observations indicated reductions in ecosystem functions at concentrations that are assumed being protective in regulation. However, high variation within and between studies hampered the derivation of a concentration-effect relationship and thus effect thresholds.

In chapter 3, I conducted a field study to determine the microbial and invertebrate-mediated OMB by deploying fine and coarse mesh leaf bags in streams with forested, agricultural, vinicultural and urban riparian land use. Additionally, physicochemical, geographical and habitat parameters were monitored to explain potential differences in OMB among land use types and sites. Regarding results, only microbial OMB differed between land use types. The microbial OMB showed a negative relationship with pH while the invertebrate-mediated OMB was positively related to tree cover. OMB responded to stressor gradients rather than directly to land use.

In chapter 4, macroinvertebrates were sampled in concert with leaf bag deployment and after species identification (i) the taxonomic diversity in terms of Simpson diversity and total taxonomic richness (TTR) and (ii) the functional diversity in terms of bio-ecological traits and Rao's quadratic entropy was determined for each community. Additionally, a land-use gradient was established and the response of the taxonomic and functional diversity of invertebrate communities along this gradient was investigated to examine whether these two metrics of biodiversity are predictive for the rate of OMB. Neither bio-ecological traits nor the functional diversity showed a significant relationship with OMB. Although, TTR decreased with increasing anthropogenic stress and also the community structure and 26 % of bio-ecological traits were significantly related to the stress gradient, any of these shifts propagated to OMB.

Our results show that the complexity of real-world situations in freshwater ecosystems impedes the effect assessment of chemicals and land use for functional endpoints, and consequently our potential to predict changes. We conclude that current safety factors used in chemical risk assessment may not be sufficient for pesticides to protect functional endpoints. Furthermore, simplifying real-world stressor gradients into few land use categories was unsuitable to predict and quantify losses in OMB. Thus, the monitoring of specific stressors may be more relevant than crude land use categories to detect effects on ecosystem functions. This may, however, limit the large scale assessment of the status of OMB. Finally, despite several functional changes in the communities the functional diversity over several trait modalities remained similar. Neither taxonomic nor functional diversity were suitable predictors of OMB. Thus, when understanding anthropogenic impacts on the linkage between biodiversity and ecosystem functioning is of main interest, focusing on diversity metrics that are clearly linked to the stressor in question (Jackson et al. 2016) or integrating taxonomic and functional metrics (Mondy et al., 2012) might enhance our predictive capacity.

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Abbriviations

App.	Appendix
B – EF	Biodiversity – Ecosystem Function
BIC	Bayesian Information Criterion
CLC	Corine Land Cover
EC	Electrical Conductivity
EC_{50}	Median Effect Concentration
EQS	Environmental Quality Standard
EU	European Union
FD	Functional Diversity
Log TU	Logarithmic sum of Toxic Units
OMB	Organic Matter Breakdown
OMB_{macro}	Invertebrate-mediated Organic Matter Breakdown
OMB_{micro}	Microbial Organic Matter Breakdown
PP	Primary Production
RQE	Rao’s Quadratic Entropy
SD	Simpson Diversity
TTR	Total Taxonomic Richness
TU	Toxic Unit
UP	Uniform Principles
WFD	Water Framework Directive

- Chapter 1 -

INTRODUCTION & OBJECTIVES

1.1 STREAM FUNCTIONING & BIODIVERSITY

The human population is predicted to increase from currently ~ 7.5 to around 9 billion people in 2050 with ever growing demands for space, clear drinking water, food, fiber and energy (Foley et al. 2005, MEA 2005, Godfray et al. 2010). Thus, world's ecosystems are under great pressure satisfying anthropogenic demands (MEA 2005). Although, only 0.01 % of global water corresponds to lentic and lotic freshwaters, such as lakes, rivers/streams and reservoirs (Shiklomanov 1993), they provide a great variety of crucial services to human society: domestic use, irrigation, power generation, transport, clean drinking water or fishery products (Palmer et al. 2004). The supply of such ecosystem services depends essentially on ecosystem functions (MEA 2005), where a single service can depend on several ecosystem functions or a single function can contribute to several services (Costanza et al. 1997). From an ecological perspective, ecosystem functions are simply the level, rate or temporal dynamic of ecological processes that maintain the ecosystem (Tilman 2001, Jax 2005). However, from an anthropocentric perspective it is the capacity of ecological processes to provide goods and services to human society (De Groot et al. 2002), categorized in four groups: (1) regulation functions (e.g. water regulation), (2) habitat functions (e.g. refugium functions), (3) production functions (e.g. food or genetic resources) and (4) information functions (e.g. recreation or aesthetic information; cf. also with (TEEB 2010)).

In freshwaters, organic matter breakdown (OMB) and primary production (PP) are fundamental ecosystem functions. Depending on the location within the river network the contribution of OMB and PP in the overall energy supply varies (Webster 2007). OMB represents the most important energy source in terms of organic carbon in the first ten kilometers from the stream source, especially in forested headwaters (first to third order streams; Wallace et al. 1997). OMB is defined as the heterotrophic process of breaking down allochthonous organic matter (i.e. leaves or woody debris) into its inorganic constituents (e.g. carbon dioxide, inorganic nitrogen and phosphorus) from physical fractionation, leaching of soluble compounds, microbial (fungal and bacterial) conditioning and invertebrate feeding (Tank et al. 2010). When organic matter from the riparian vegetation enters the stream it is rapidly colonized and degraded by aquatic fungi and bacteria. During this process fungi and bacteria produce enzymes transforming inedible into edible plant components that can be assimilated by invertebrate detritivores (i.e. shredders; Graca 2001). Generally, fungi dominate the microbial breakdown process (e.g. Abelho 2001) albeit similar contribution of bacteria and fungi has been reported occasionally (Hieber and Gessner 2002). After organic matter is colonized by microorganisms shredders start feeding on it, as they prefer leaves conditioned by microorganisms over unconditioned leaves (Graca 2001). Shredders are typically aquatic insects for instance of the genera *Plecoptera* or *Trichoptera* as well as crustaceans of the genus *Gammarus* (Tachet et al. 1987). By feeding on the leaf material they shred the material and produce fine particulate organic matter (FPOM). Accrued FPOM and gained biomass not only fuel local but also subsidize downstream food webs (Webster 2007). Moreover, the fact that more than 80 % of the river network in Europe consists of small rivers and streams (Kristensen and Globevnik 2014) emphasizes the importance of the OMB even more. Regarding the contribution of microorganisms and invertebrates to the OMB, invertebrates can largely exceed the decomposer activity compared to microorganisms (Hieber and Gessner 2002). However, results can be contrasting and contributions can differ due to climate, hydrology or invertebrate density (Graça et al. 2015). Streams of higher order (i.e. fourth to sixth order) are characterized by greater width with less shading by riparian vegetation. Thus, downstream sections receive more solar radiation and the autotrophic process of primary production becomes increasingly important (Webster 2007). By means of photosynthesis, aquatic plants, algae, or bacteria convert inorganic compounds (water, nutrients and carbon dioxide) into complex organic molecules (also termed primary production). During this process biomass is metabolized, which in turn delivers energy to heterotrophic consumers (e.g. grazers or scrapers). Moreover, in terms of detritus plant material delivers energy for heterotrophic organisms, such as decomposers (Allan and Castillo 2009). Furthermore, as a part of the gross primary production (GPP, i.e. total amount of fixed carbon by autotrophic organisms) and heterotrophic fixation, respiration is also a pivotal process that provides energy to stream organisms by metabolizing organic carbon (Solomon et al. 2013). With increasing stream size PP replaces OMB as the fundamental source of energy. Yet, rivers of orders higher than 6 mainly obtain their energy from upstream organic input, river plankton and floodplains (Allan and Castillo 2009).

As outlined above, ecosystem functions (such as OMB and PP) are largely regulated by a range of organisms. Investigations regarding the fundamental relationship between organism's diversity or community composition and ecosystem functioning grew enormously in the early 1990's – soon after the Earth Summit in Rio de Janeiro. Scientists were increasingly interested in how changes in the diversity of organisms can affect ecosystem functioning and hence affect human society (Naeem et al. 2009, Cardinale et al. 2012, Gamfeldt et al. 2012). Although greater species diversity most likely benefits ecosystem functions, the relationship between biodiversity and ecosystem functioning (B-EF) is highly complex and underlies several different mechanisms (Loreau and Hector 2001, Cardinale 2011, Cardinale et al. 2011, Ebeling et al. 2014). While mechanisms, such as complementarity or the selection effect, are responsible for a positive B-EF relationship, a loss in species can also be indifferent for ecosystem functioning due to functional redundancy (Hooper et al. 2005, Fetzer et al. 2015). Nevertheless, the loss of species with key functional roles most likely results in a reduction in ecosystem functions (Cardinale et al. 2006). Thus, organismal traits play a crucial role in influencing ecosystem functions (Chapin et al. 2000) as they determine the direction and magnitude of changes in ecosystem functioning (Cadotte et al., 2011, Tolkkinen et al. 2013). This emphasizes the complexity of B-EF relationships, especially since biodiversity is beyond species diversity and ranges from genetic diversity within populations to community diversity across landscapes (Sala et al. 2000), including functional diversity (FD; Cadotte et al. 2011). FD refers to the distribution of species and their abundance within the functional space, including functional richness, functional divergence and functional evenness as three primary components (Mason et al. 2005).

Thus, when ecosystem goods and services are to be preserved for present and future generations we need a better understanding of the complex B-EF relationship. Regarding freshwater-related services, this means exploring the relationship between FD and the OMB is of central importance (Frainer and McKie 2015, Tolkkinen et al. 2015). The loss in freshwater biodiversity is faster than in any other of the world's major biomes (Reynolds and Souty-Grosset 2012). Hence, identifying threats and stressors and quantifying their effects on biodiversity and ecosystem functioning, would be a first step towards informing the preservation of ecosystem services (Covich et al. 2004, Hooper et al. 2005, Díaz et al. 2006, Naeem et al. 2009).

1.2 THREATS TO STREAM FUNCTIONING & BIODIVERSITY

The Millennium Ecosystem Assessment (MEA 2005) has identified several factors (most of them related to anthropogenic activities) jeopardizing stream ecosystem functions and the biodiversity supported by freshwaters (that makes up nearly 6 % of global biodiversity; Dudgeon et al. 2006). Economic, technological and socio-political forces have been identified as underlying causes of ecosystem changes and the loss in ecosystem services, with agriculture, energy and resource extraction, traffic and the development of infrastructure being of central importance (MEA 2005). In this context, the land surface has been transformed to a large proportion, chemicals have been released in great amounts and even the climate system has started to change (recent rate of global warming has been unprecedented in the past 1,300 years; Foley et al. 2005, MEA 2005, IPCC 2007).

As a result of increasing anthropogenic land use, natural ecosystems are becoming intensely fragmented by roads, railway lines, buildings and agricultural areas (Ellis et al. 2006).

According to the United Nations Environment Programme (UNEP 2014), roughly 2 % of land worldwide are covered by urban areas (i.e. infrastructure and cities) and around 33 % by agricultural areas (e.g. pasture, cropland, viniculture) – representing the largest use of land on earth. Globally, agriculture accounts for almost 70 % of all water consumption (FAO 2006). However, stream biodiversity and functioning are not only threatened by the vast needs of water for irrigating agricultural areas, resulting in water withdrawal and diversion (Foley et al. 2005) but also by factors, such as the degradation of water quality, hydromorphological changes (e.g. channelization of streams or dams), logging the riparian vegetation or species invasion (MEA 2005, Dudgeon et al. 2006, Schwarzenbach et al. 2010, Vörösmarty et al. 2010). These factors are related to a variety of stressors, i.e. shifts in abiotic or biotic variables that exceed the range of normal variation and adversely affect individual physiology or population performance in a statistically significant way (Auerbach 1981). Various distinct transgressions of normal, abiotic variation can be identified (MEA 2005), for instance (i) nutrient enrichment due to fertilizer use in agricultural areas (Schwarzenbach et al. 2010), (ii) secondary salinization or inputs of heavy metals due to deicer for roadways or mining, respectively, in urban areas (Hogsden and Harding 2012, Cañedo-Argüelles et al. 2013) or (iii) homogenizations of hydraulic conditions due to stream channelization in agricultural and urban areas (Poff et al. 2007). In this context, the pollution by pesticides in agricultural areas is the embodiment of a stressor, as pesticides are intentionally applied to control pests and weeds. Thus, nutrient enrichment, secondary salinization, heavy metals and pesticides can be interpreted as land use associated stressors. Large amounts of chemicals (e.g. nutrients or pesticides) enter streams via diffuse/non-point (e.g. spray drift or surface runoff) or point sources (e.g. industrial discharge, mine waste water or sewage plants; Schulz 2004, Creusot et al. 2014, Sims et al. 2013)). Increasing applications of nitrogen and phosphate fertilizers related to agricultural food production (by ~638 % & and 203 %, respectively, between 1961 and 1999; Green et al. 2005)), are responsible for elevated nitrogen and phosphate concentrations frequently found in streams (Woodward et al. 2012). Similarly, pesticides' occurrence is widespread in streams (Malaj et al. 2014, Stehle and Schulz 2015b) as up to 2.5 million tons of active pesticide components out of 70,000 different chemicals are in daily use in the United States or Europe (Schwarzenbach et al. 2006, Fenner et al. 2013).

Anthropogenic stressors can individually or jointly compromise the biodiversity of species in streams (Dudgeon et al. 2006) and ecosystem functioning (Jonsson et al. 2002), depending on species functional traits. For instance, nutrient enrichment but also secondary salinization can reduce stream biodiversity and alter ecosystem functioning (e.g. Cañedo-Argüelles et al. 2013, Herbert et al. 2015). Regarding OMB, low levels of nutrient enrichment can accelerate the breakdown process (Ferreira et al. 2015), while exceeding nitrate concentrations of approximately 10 mg/L reduce OMB (Woodward et al. 2012). Secondary salinization can adversely affect breakdown rates in streams (Gómez et al. 2016) while it also alters the pH in water (Herbert et al. 2015). For instance, alkaline conditions can cause reductions of the stream biodiversity (Casas and Descals 1997), whereas contrasting results have been found regarding propagation to reduced OMB (Dangles and Chauvet 2003, Niyogi et al. 2013). Moreover, most inland water bodies have been irreversibly hydromorphologically transformed, channelized and dredged (Lévêque and Balian 2005). These modifications of the erosion and sedimentation patterns (Baron et al. 2002, Poff et al. 2007) can lead to declining biodiversity and OMB (Rasmussen et al. 2012, Mollá et al. 2017). Furthermore, the Millennium Ecosystem Assessment identified heavy metals and pesticides as highly relevant in terms of threatening freshwater ecosystems (MEA 2005). However, in order to meet human demands for food, pesticides (fungicides, herbicides and insecticides) have become the key element of global agricultural pest and weed control (Reuters 2008). After agricultural pesticide application, these toxicants partly enter stream ecosystems, by e.g. runoff and pose substantial threats to non-target stream organisms (Beketov et al. 2013, Malaj et al. 2014, Stehle and Schulz 2015a) and functioning (Rasmussen et al. 2012, Schäfer et al. 2012). For instance, a meta-analysis of field studies found significant reductions in the abundance of sensitive stream organisms already at pesticide concentrations that are assumed to be protective in the Uniform Principles of the European Union for the authorization of pesticides (first tier; EEC 1991a), including adverse effects on ecosystem functioning (Schäfer et al. 2012). Thus, regulatory threshold (refer to section 1.3) established for organisms may not be protective for ecosystem functions for which they are adopted (Woodward et al. 2012). Whereas pesticides are degraded by a range of abiotic (e.g. photochemical reactions) and biotic (by microorganisms) transformations within the environment (Fenner et al. 2013) heavy metals can persist in freshwaters for a long time (Schwarzenbach et al. 2006). They enter stream ecosystems e.g. by storm water runoff from urban roadways (e.g. Zinc, Cadmium or Copper; Sansalone and Buchberger 1997) or by mine drainage (e.g. Iron or Aluminum; Niyogi et al. 2002). In streams they can accumulate in sediments and can reduce biodiversity (Fanny et al. 2013) and ecosystem functioning (Costello and Burton 2014).

In order to protect and preserve freshwater ecosystems from above mentioned stressors associated with anthropogenic land use, ecosystem health should be assessed adequately. In this context, leading scientists call for complementing current bioassessment that is mainly based on structural measures (such as water chemistry, hydrogeomorphology, and biological diversity metrics) with functional measures (such as OMB; Woodward et al. 2012). However, for both measures going beyond current implementation could improve the bioassessment and hence compose a more integrated picture of freshwater ecosystem health. Firstly, with regard to diversity metrics as structural measure, several studies have found that alterations in the FD of stream communities affected ecosystem functions (e.g. Díaz and Cabido 2001, Flynn et al. 2009, Frainer and McKie 2015), depending on organismal traits of community members as mentioned in section 1.1. However, freshwater ecosystems are still largely assessed using taxonomic diversity metrics or indicators (e.g. EPT richness, saprobic index or RIVPACS). While organismal traits allow for the link to ecosystem functioning (Cadotte et al. 2011) taxonomic indicators do not allow for a direct evaluation of the status of ecosystem functions. Moreover, traits often respond rapidly and predictably to multiple stressors (Dolédec et al. 2006, Doledec and Statzner 2010, Mouillot et al. 2013) which lead to the assumption that FD metrics could be more suitable in indicating changes of ecosystem functions than taxonomic diversity metrics (Cadotte et al., 2011). However, only few comparative studies exist in the context of effects by anthropogenic stressors (e.g. Dolédec et al. 2006, Doledec et al. 2011), especially in the context of stressor effects on ecosystem functions, such as OMB (e.g. Frainer and McKie 2015, Tolkkinen et al. 2015). Thus, **ascertaining the suitability of taxonomic and functional diversity metrics for predicting the rate of organic matter breakdown** could help to improve or complement current bioassessment and would provide additional knowledge about B-EF relationships. Secondly, beside the debate to integrate functional measures into current bioassessment the spatial scale on which data are gathered plays an important role (Dolédec and Statzner 2010). In the context of land use effects on ecosystem functions, a trend towards adverse effects on OMB might be recognizable. However, generalizations cannot be made and studies investigating this topic often provided contrasting results. For instance, Sponseller and Benfield (2001) found that breakdown rates in urban areas decreased with increasing sediment loadings, while Meyer et al. (2005) observed a positive relationship between breakdown rate and urbanization due to nutrient enrichment. Similarly, inconsistent results were reported for effects of agricultural land use (cf. Magbanua et al. 2010 vs. Piscart et al. 2009). The high variability between studies could be attributed to variable associations of stressors with specific land use types or to variable responses to stressors. Moreover, most of the previous studies were conducted on a local scale (i.e. ≤ 5 streams per land use type; but see Woodward et al. 2012) with relatively small sample sizes (total $n \leq 10$ in most studies) that could further explain inconsistent results. However, in order to adequately protect and preserve freshwater ecosystems from anthropogenic land use and its associated stressors, quantifications of potential effects on a regional scale are highly relevant (Dolédec and Statzner 2010). This is because anthropogenic stressors operate over multiple spatial scales (Allan 2004) and individual studies on local scales do not allow for (i) comparison of results within or among land use types and (ii) extrapolation of land use effects to larger spatial scales. Extrapolation would require the establishment of a link between land use and ecosystem function on the respective scale. Hence, **studies are needed that include the assessment of ecosystem functions on a regional scale** (Vighi et al. 2006). Consequently, a regional overall picture would help understanding the extent of influence by different stressors for the OMB. Hence, it creates a basis for a sustainable treatment of the resource water, as required for the Water Framework Directive (EC 2000).

In order to assess the relevance of heavy metals and pesticides regarding their impact on ecosystem functions studies are needed that can be analyzed in a comparative way. However, existing reviews on the effects of land use associated stressors on ecosystem functioning have either exclusively addressed herbicide or heavy metal effects on ecosystem functions (Brock et al. 2000, Ferreira et al. 2016) or solely considered pesticide effects on microbial community respiration and net primary production as ecosystem functions (DeLorenzo et al. 2001). **A comprehensive study addressing effects of several stressors on several ecosystem functions is needed** to quantify their overall impact or reveal general patterns. This can assist in the understanding of the potential threat by anthropogenically-induced stressors for freshwater ecosystem functions.

1.3 LEGAL FRAMEWORKS FOR FRESHWATER PROTECTION

A more sustainable freshwater management is needed to manage the alarming effects of land use and its associated stressors on stream ecosystem functioning (Lepom and Hanke 2008). Various strategies exist in order to protect streams including recycling or retention of runoff water, conservation farming methods, improved agricultural land management and the use of buffer strips and riparian vegetation for filtration (Bowmer 2013). In this context, obligatory European legal frameworks or directives focusing on freshwaters are for instance: the drinking water directive (EC 1998), the urban waste water treatment directive (EEC 1991b), the nitrates directive (EEC 1991c), the directive for the sustainable use of pesticides (EC 2009a) or the water framework directive (WFD; EC 2000). Particularly the WFD aims at the protection and enhancement of the status of aquatic ecosystems by achieving a good ecological and chemical status of European surface waters (EC 2000). A good status is defined as a minimum of anthropogenic impact, i.e. no or only minor hydromorphological changes (hydraulic engineering projects) and chemical pollution (waste water or nutrients). Regarding the ecological status, quality assessment focuses on biological quality elements, such as the composition and abundance of aquatic flora (including diatoms and macrophytes), invertebrates and fish fauna (EC 2000). Regarding the chemical status as defined in the WFD, the main aim is that field chemical concentrations should not exceed environmental quality standards (EQS) for substances that have been classified as priority substances (EC 2000, 2013). Another strategy to protect the environment aims at identifying and predicting the risk of chemicals before application. The so–called risk assessment for each chemical (e.g. a plant protection product) must be evaluated before its authorization (Suter 2008, EC 2009b). Generally, the risk assessment follows a tiered approach (EFSA, 2013), with the first tier investigating the response of standard test organisms to acute or chronic toxicant doses under laboratory conditions (dose – response relationship). However, depending on the toxicity test and on the substance (e.g. insecticides or herbicides) the standard test organisms can differ, mostly representing different trophic levels: algae (e.g. *Pseudokirchneriella subcapitata*), arthropod (e.g. *Daphnia magna*) and fish (e.g. *Pimephales promelas*) but for insecticides, for instance, an additional test species (e.g. *Chironomus sp.*) is required (EFSA 2013). Regarding the dose-response relationship, reference is typically made to fixed values, such as EC_{50}: where 50 % of the tested population suffers a particular effect (e.g. LC_{50} refers to the concentration at which 50 % of the population suffers lethal effects; Calow and

Forbes 2003). Concerning for instance pesticides: the Uniform Principles (UP) of the European Union (EU) assume in the first tier for the authorization of pesticides (EEC 1991a) that exposures lower than 1/100 and 1/10 of the median effect concentration (EC_{50}) for two standard test organisms (*Daphnia magna* and *Pseudokirchneriella subcapitata*) should have no or no unacceptable adverse effects on freshwater organisms. This corresponds to a toxic unit (TU; Sprague 1970) of 0.01 and 0.1, and reflects a safety factor of 100 and 10, respectively.

The knowledge about the protectiveness of these adopted thresholds for ecosystem functioning is still incomplete and **effect thresholds that are protective also for functional endpoints are needed** in order to adequately protect stream ecosystems.

1.4 OBJECTIVES & STRUCTURE OF THE THESIS

Overall, in this thesis I aimed at contributing to a better understanding of human impacts on the environment, with a particular emphasis on how anthropogenic land use and associated stressors jeopardize freshwater biodiversity and functioning.

In detail, the thesis addresses the following three research objective (see Figure 1.1):

- Assessing the relevance of pesticides and heavy metals regarding their influence on three ecosystem functions (organic matter breakdown, primary production and community respiration) by identifying effect thresholds based on a literature review (chapter 2, Appendix A).

- Quantifying the impact of three land-use types (agriculture, viniculture and urbanization) on a fundamental ecosystem function on a regional scale. The conducted regional field study (in streams of 26 catchments) assesses the relevance of anthropogenic land use regarding its impact on the organic matter breakdown (chapter 3, Appendix B).

- Quantifying the impact of anthropogenic land use (in terms of a stressor gradient) on the taxonomic and functional diversity of invertebrate communities (based on the above mentioned regional field study) and ascertaining the suitability of these two biodiversity metrics for predicting the rate of organic matter breakdown (chapter 4 and Appendix C).

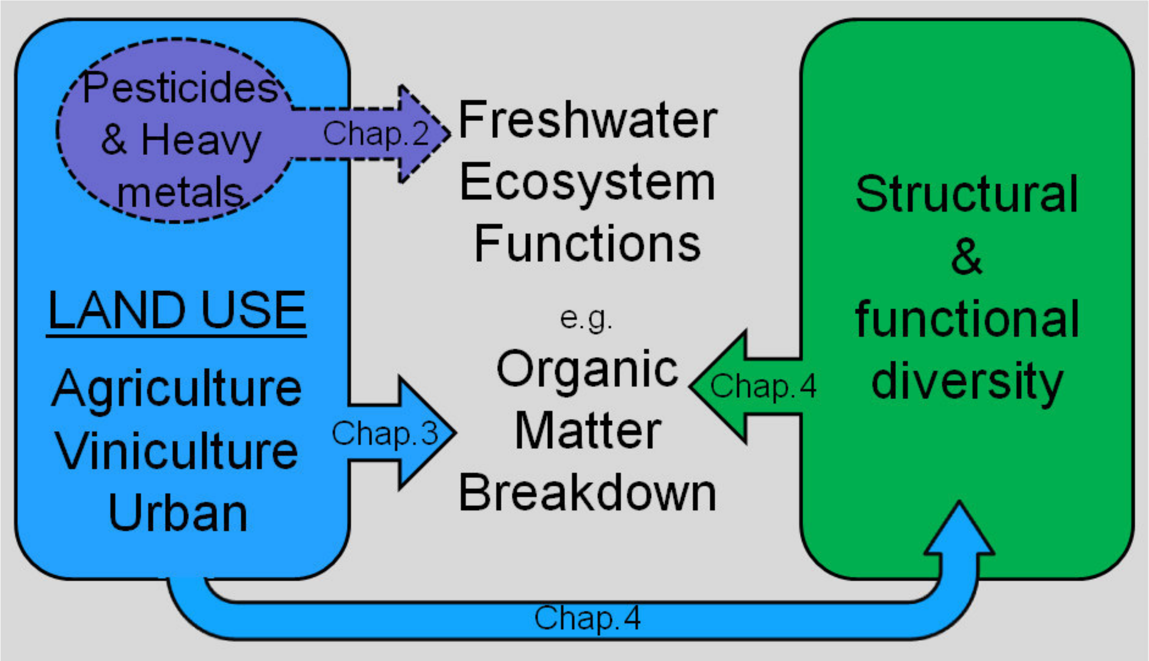

Fig. 1.1: Overview of this thesis based on the thematic background presented in the introduction (see chapter 1). Summary of: the three research objectives (chapter 2-4), the sources of impact (indicated by different colors) and the main method used (indicated by different line types). The impact on either three ecosystem functions (chapter 2) or exclusively on the organic matter breakdown (chapter 3 and 4) was determined. Purple: effects due to pesticides and heavy metals as land-use associated toxicants; blue: effects due to three land use categories (chapter 3) or a land-use gradient (chapter 4); green: effects due to changes in invertebrate biodiversity. Dashed lines: literature review, solid lines: field study (details within the respective chapters).

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- Chapter 2 -

REVIEW ON THE EFFECTS OF TOXICANTS ON FRESHWATER ECOSYSTEM FUNCTIONS

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2.1 ABSTRACT

We reviewed 122 peer-reviewed studies on the effects of organic toxicants and heavy metals on three fundamental ecosystem functions in freshwater ecosystems, i.e. leaf litter breakdown, primary production and community respiration. From each study meeting the inclusion criteria, the concentration resulting in a reduction of at least 20% in an ecosystem function was standardized based on median effect concentrations of standard test organisms (i.e. algae and daphnids). For pesticides, more than one third of observations indicated reductions in ecosystem functions at concentrations that are assumed being protective in regulation. Moreover, the reduction in leaf litter breakdown was more pronounced in the presence of invertebrate decomposers compared to studies where only microorganisms were involved in this function. High variability within and between studies hampered the derivation of a concentration-effect relationship. Hence, if ecosystem functions are to be included as protection goal in chemical risk assessment standardized methods are required.

2.2 INTRODUCTION

The Millennium Ecosystem Assessment identified anthropogenic toxicants as a major threat for freshwater ecosystems (MEA, 2005), with pesticides and heavy metals being considered as most relevant. Both enter aquatic ecosystems via various paths such as mine waste water, industrial discharge, drainage, spray drift or runoff (Sierra and Gomez, 2010, Niyogi et al., 2002, Arts et al., 2006, Gjessing et al., 1984) and may in turn affect aquatic communities (e.g. Beasley and Kneale, 2003, Clements et al., 2000, Schäfer et al., 2011a, Liess et al., 2008, Widenfalk et al., 2008). To protect aquatic ecosystems, the Uniform Principles (UP) of the European Union (EU) require for the first tier in the authorization of pesticides that the pesticide exposure should be lower than 1/100 and 1/10 of the median effect concentration (EC50) for *Daphnia magna* and *Pseudokirchneriella subcapitata* (EEC, 1991), respectively. This corresponds to a toxic unit (TU; Sprague, 1970) of 0.01 and 0.1, and reflects a safety factor of 100 or 10, respectively. While the suitability of extrapolating effects on ecological communities from standard test organisms has been questioned (Cairns, 1986; Rubach et al., 2010), in retrospective risk assessment data are often limited to these test organisms (Strempel et al., 2012) and they are consequently used to standardize the risks from different toxicants.

By applying the abovementioned safety factors, concentrations below these thresholds are assumed to cause no or no unacceptable adverse effects on macroinvertebrates and algae, respectively.

In this context, a review of mesocosm studies on several pyrethroid, organophosphate and carbamate insecticides reported that a TU of 0.01 for the most sensitive species, which was *D. magna* in most cases, did not cause notable effects in freshwater communities (Van Wijngaarden et al., 2005). By contrast, a meta-analysis of field studies on pesticide effects showed that TUs 10 to 100-fold below the UP lead to a significant reduction in the abundance of sensitive macroinvertebrate taxa (Schäfer et al., 2012b). As structural alterations can compromise ecosystem functioning (Doledec et al., 2006, Gücker et al., 2006), the observed decrease in sensitive taxa was hypothesized to be the cause of the reported reduction in invertebrate-mediated leaf litter breakdown (Schäfer et al., 2012b). Thus, the UP thresholds for structural endpoints may not be protective for ecosystem functions (cf. Woodward et al., 2012), though no reduction in primary production and community respiration was found for a pesticide gradient ranging from a $TU_{\textit{D. magna}}$ of 0.1 to 0.001 in 24 South-East Australian streams (Schäfer et al., 2012a).

Overall, reductions in leaf litter breakdown and primary production are of particular concern because these functions represent the main energy sources for local and downstream freshwater food webs (Wallace et al., 1997, Webster, 2007). While microbial decomposers and invertebrate detritivores degrade and shred leaf material, respectively (i.e. leaf litter breakdown; Graca, 2001, Hieber and Gessner, 2002), algae and macrophytes are the main groups responsible for the conversion of sunlight into biomass via photosynthesis.

Recent reviews mainly focused on heavy metal (Fleeger et al., 2003) or pesticide (Brock et al., 2000b, Brock et al., 2000a, Van Wijngaarden et al., 2005) effects on community structure whereas ecotoxicological effects on ecosystem functions in lotic and lentic ecosystems have been largely ignored – with two exceptions: while Brock et al. (2000a) exclusively discussed herbicide effects on ecosystem functions, the review of De Lorenzo et al. (2001) was restricted to effects of pesticides on microorganisms, only considering the functions of community respiration and net primary production. In the present study effects of toxicants on three fundamental ecosystem functions (i.e. leaf litter breakdown, primary production and community respiration) are considered. Thereby, we aimed at identifying effect thresholds based on the relationship between ecosystem functions and standardized concentration-effect relationships. In this context, the second aim was to examine whether effects of organic toxicants on functional endpoints occur below thresholds of the UP. Finally, given that macroinvertebrates belong to the most sensitive group of organisms with regard to organic toxicants (Schäfer et al., 2011b), we hypothesized that ecosystem functions involving invertebrates (e.g. leaf litter breakdown) are more sensitive than those that do not (e.g. primary production or microbial respiration).

2.3 MATERIAL AND METHODS

2.3.1 LITERATURE SELECTION

The databases “Web of Knowledge” and “Pubmed” were searched for publications on the effects of toxicants on three ecosystem functions, i.e. leaf litter breakdown, primary production and community respiration. The search was limited to articles published between January 1980 and March 2012. The databases were queried by combining different terms for freshwater ecosystems (freshwater* OR stream* OR river* OR pond* OR lake*) supplemented by terms specifying the toxicants (chemical* OR contaminant* OR pollutant* OR toxicant* OR pesticide* OR heavy metal* OR metal* OR fungicide* OR herbicide* OR insecticide*) and ecosystem functions (ecosystem function* OR primary product* OR respiration* OR leaf litter breakdown OR decomposition*) of interest. Moreover, the reference lists of identified articles were inspected for further literature. Given that our review focuses on lotic and lentic freshwater systems, publications regarding the influence of toxicants on ecosystem functions in the marine system, marsh land, coastal waters or groundwater were excluded. Also, investigations on eutrophication (10-fold higher nutrient load than the control) and acidification (pH < 5) were omitted irrespective whether originating from human activities or natural processes because both conditions may lead to dramatic changes in the ecosystems (Jüttner et al., 2010; Ormerod and Durance, 2009) and would be indistinguishable from toxicant effects. Finally, in situations where multiple studies relied on the same raw data, only the study providing the most complete required information (chapter 2.3.2 Minimum effect size) was considered. An overview of all reviewed and excluded studies is given in the Supplementary data (Table A.1, A.2 in App. A).

2.3.2 MINIMUM EFFECT SIZE

The identified studies were grouped regarding the investigated toxicant: (1) heavy metals, (2) organic toxicants, and (3) miscellaneous (i.e. sodium hypochloride, and a mixture of cadmium and phenanthrene). The latter group comprised only two studies and was thus not considered in further analyses. The group of organic toxicants was further subdivided into fungicides, insecticides, herbicides, pharmaceuticals, pesticide mixtures and others (i.e. phenolic compounds and polycyclic aromatic hydrocarbons; App. A, Tab. A.1). To derive a suitable effect concentration (EC) (in µg/L), we first determined the relative mean standard deviation (RMSD) for reference sites/control treatments for studies on the most frequently assessed ecosystem function (leaf litter breakdown). This was calculated as approximately 12%. To discriminate true effects from noise in terms of RMSD while retaining sensitivity to detect effects, the effect size considered for this review was set to ≥ 20%, which did not result in a bias against studies with brief or episodic exposures (cf. App. A, Tab. A.3). Therefore, the EC causing a reduction of ≥ 20 % in an endpoint related to an ecosystem function was selected as basis for all further analyses. From each study only one effect on functional endpoints per observation was extracted, i.e. once the minimum effect size was reached or exceeded. For studies on leaf litter breakdown, the effect size referred to breakdown rates or mass loss as endpoints, whereas for (gross) primary production it referred to the amount of fixed carbon, as well as oxygen production. For community respiration the amount of carbon consumed or oxygen produced was used as endpoint. Studies only reporting dissolved oxygen (DO) were excluded, since net DO can originate from multiple sources, such as aquatic plants and the ambient atmosphere, simultaneously. Additionally, five studies reporting hormesis-like effects (Calabrese and Baldwin, 1998) were omitted, since our review focused on adverse effects and an increase in one endpoint does not necessarily indicate improved ecological health (Kefford et al., 2008) or may be an indirect effect of a non-measured adverse effect (Preston, 2002).

2.3.3 EXPLANATORY VARIABLES

Beside TU and a dummy variable coding the group of toxicants (i.e. heavy metals, organic toxicants, miscellaneous), five additional variables (I-V) were included to explain the variability in the functional endpoints. First, each observation derived from an included study was categorized with respect to the (I) group of organisms that provides the according ecosystem function: (a) microbial decomposer community (i.e. bacteria and fungi), (b) decomposer-detritivore community (i.e. macroinvertebrates and microorganisms), and (c) aquatic plants (i.e. phytoplankton, macrophytes, etc.). We followed the definitions of communities as described in the original studies. Note that for leaf decomposition, the communities are defined based on litter bag mesh size, which can differ between studies (Pye et al., 2012). Second, the observations were classified according to (II) ecosystem type – (a) lotic and (b) lentic – and to (III) study system: (a) field, (b) semi-field studies (i.e. mesocosm, artificial streams, etc.), and (c) laboratory (i.e. microcosm experiments). We note that except for field studies, rather community than ecosystem functions are measured. However, to enhance readability the term ecosystem function is used for all studies. Moreover, the (IV) exposure scenario, either (a) episodic or (b) chronic, was included as explanatory factor. Episodic exposure refers to single applications of toxicants in laboratory studies or individual run-off events in field studies. The included studies did not feature multiple exposure scenarios. Chronic exposure refers to relatively constant concentration of toxicants under laboratory or field (e.g. mine waste water) conditions (App. A, Tab. A.1). Finally, the exposure time (V) was determined as continuous variable (in days), i.e. the period until the minimum effect size of 20% was reached or exceeded (App. A, Tab. A.1).

2.3.4 CALCULATION OF TOXIC UNITS

Comparing the effects from different toxicants requires a benchmark. Ideally, this would be related to the ecosystem function under scrutiny, for example EC(x) values of the different toxicants for the ecosystem function that were produced under standard laboratory conditions. Since such data are not available, we reverted to ecotoxicological standard test organisms to

compare the toxic effects from different stressors. This procedure was successfully employed in recent studies on ecotoxicological effects on ecosystem functions (Rasmussen et al., 2012, Schäfer et al., 2012b). We note that this only serves the purpose to establish a basis for comparison of different toxicants but is by no means intended to suggest that these organisms would play a crucial role in the respective function. *D. magna* was selected as standard test organism for ecosystem functions provided by invertebrates. *P. subcapitata* was selected for ecosystem functions performed by aquatic plants or microorganisms, because only very few EC50 values for e.g. fungi were available (cf. Rasmussen et al., 2012; Schäfer et al., 2011a). However, if the required information was not available for *P. subcapitata* (see below) other algae species (e.g. *Raphidocelis subcapitata*) were selected. This was the case for ten toxicants (App. A, Tab. A.4).
The logarithmic sum of toxic units (log TU) was calculated as follows:

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

where c represents the concentration (µg/L) of each toxicant i, $EC50_i$ is the median effect concentration of the respective toxicant i from standard laboratory toxicity tests and n gives the number of toxicants that caused a ≥ 20 % reduction in the respective ecosystem function. EC50 values were taken from the ECOTOX (USEPA, 2012), Pesticide Properties (FOOTPRINT, 2011) and/or Veterinary Substances (VSDB, 2011) databases (App. A, Tab. A.4). An exposure time of 48 hours was selected or the nearest exposure time for toxicants where no data for 48-h was available (App. A, Tab. A.4). Furthermore, when more than one EC50-value was available the arithmetic mean was calculated. Since the first tier of the UP for pesticide authorization employ *D. magna* and algae as benchmark organisms, our TUs for pesticides are directly comparable to this regulatory threshold of 0.01 and 0.1, respectively (EEC, 1991). Moreover, we adopted the TU of 0.1 for microbial biota. A corresponding threshold does not exist for heavy metals, though environmental quality standards (EQS) have been established. These EQS consider important determinants of metal toxicity in a site such as the chemical speciation of metals, their bioavailability and the background concentration of metals (cf. Bass et al., 2008; EC, 2000). In addition, EQS integrate different protection goals and rely on toxicity data from different trophic levels, which further decreases their suitability as benchmark for the risks from different compounds regarding one endpoint. Hence, the results for metals are only described and not related to regulatory thresholds.

2.3.5 DATA ANALYSIS

Data analysis was performed separately for each ecosystem function, with its percentage reduction as relative response variable and including the explanatory variables outlined in chapter Explanatory variables. These variables were log- or double square-root transformed in case of strong deviation from the normal distribution, which was evaluated based on visual inspection. Linear models were established for each ecosystem function. We conducted automatic stepwise model building, starting with the null model or a reduced model containing one variable expected to be relevant and defining the null model (no explanatory variable included) as lower and the full model (all explanatory variables included) as upper limit. The statistical procedure was backward and forward entering of variables with Bayesian Information Criterion (BIC) as stepwise model selection criterion (Schwarz, 1978). Model checking included homogeneity of variance as well as normal distribution of model residuals and identification of influential observations using residual-leverage plots and Cook`s distances. Observations more than ± 2 standard deviations from the mean and/or a Cook`s distance ≥ 0.5 were omitted and the model was refitted. All calculations and graphics were done in R 2.15.2 (R Development Core Team, 2012) and the R script is available in Supplementary data (Script.R).

2.4 RESULTS AND DISCUSSION

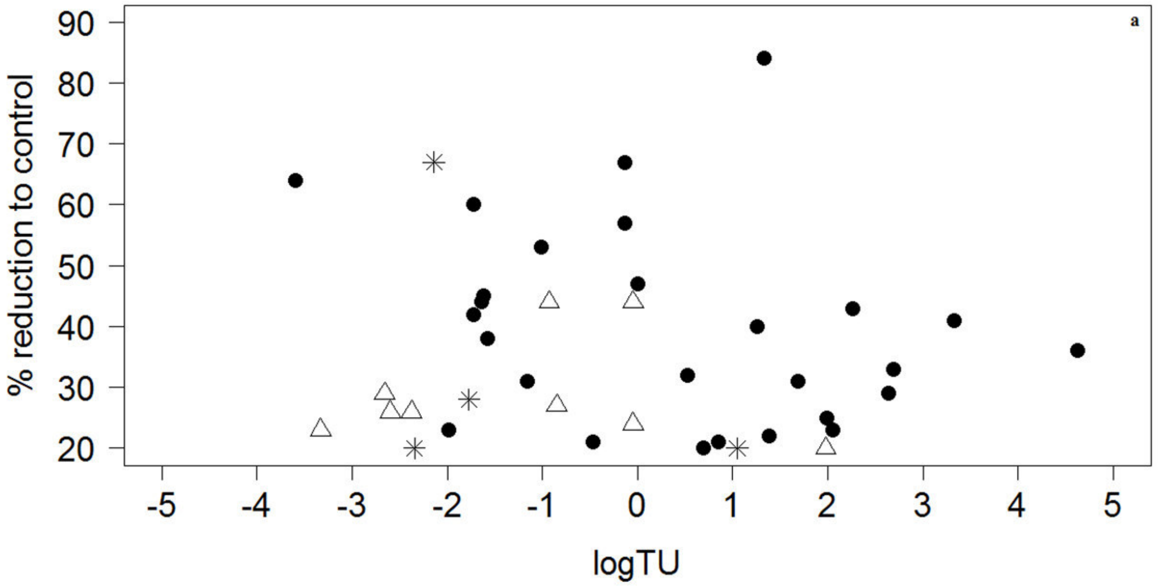
2.4.1 OVERVIEW ON STUDIES AND OBSERVED EFFECTS

A total of 122 studies on the effects of toxicants on ecosystem functions in lotic and lentic systems were found. Out of these, 76 studies were not considered, as they did not meet our selection criteria, e.g. no effect size was extractable, effects were found under acidic or elevated nutrient conditions or no control treatment was available (App. A, Tab. A.2). Therefore, this review focuses on 46 studies with a total of 75 observations. With 48 observations, the majority of studies were related to toxicant effects on leaf litter breakdown. Reductions in this ecosystem function may be attributed to a decline in feeding activity and/or mobility of macroinvertebrates, as suggested by Forrow and Maltby (2000) and Rasmussen et al. (2008). Although primary production is also an important energy source for freshwater ecosystems, studies on potential adverse effects of toxicants on this ecosystem function are rare (14 observations). The same holds for community respiration with 13 observations. The highest reduction in leaf litter breakdown (84%) and microbial community respiration (44%) was caused by heavy metal exposure originating from copper (Sridhar et al., 2001) and gold mining (Medeiros et al., 2008), respectively. An insecticide (bifenthrin) caused the highest reduction in primary production (75%; Hoagland et al., 1993). This effect may be explained by the high direct toxicity of this particular substance towards algae (Lal, 1984).

As mentioned above, *D. magna* was selected as standard test organism for invertebrate-related ecosystem functions and *P. subcapitata* was selected for ecosystem functions related to aquatic plants and microorganisms. The studies reporting effects for heavy metals spanned a logTU range of -3.6 to 4.6 (Figure 2.1a), whereas organic toxicants showed effects at a logTU range of -5.2 to 2.3 (Figure 2.1b). Since for both organic toxicants and heavy metals acute toxic effects on the most sensitive species can be approximated to occur up to 100-fold below effects on *D. magna* (Von der Ohe and Liess, 2004), which corresponds to a logTU of -2, we expected a similar effect ranges in invertebrate mediated ecosystem functions for both toxicant groups. For pesticides two observations described reductions in leaf litter breakdown (20% and 57%) below a logTU of -2 (i.e. -2.7 and -2.11, respectively). Similarly, one observation for heavy metals (64% at a logTU of -3.6) described effects below a logTU of -2. For community respiration, four observations showed effects of heavy metals (23% to 29%) at logTUs of -3.3 to -2.7. Thus, despite metal toxicity in the field being strongly influenced by various factors such as bioavailability and speciation, metals can generally impact ecosystem functions at similarly low trace levels as organic toxicants.

For pesticides, more than one third (12 observations) of 30 observations indicated reductions in one of the ecosystem functions at TUs below the UP thresholds. Of these, two observations indicated effects up to 5 times below the threshold of 0.01, and 10 indicated effects up to 17,000 times below the threshold of 0.1 (Figure 2.1b).

Our findings suggest that the three ecosystem functions considered can be adversely affected by pesticides at concentrations up to 1000-fold below TUs of 0.01 and 0.1 for *D. magna* and *P. subcapitata*, respectively. This is in agreement with a previous meta-analysis that reported effects on the macroinvertebrate community structure at a similar TU range (Schäfer et al., 2012b). However, since community data were not available for the majority of studies covered in the present review, we could not assess whether functional effects below the UP thresholds were associated with effects on structural endpoints. Hence, for pesticides it remains open, whether a protective threshold for structural endpoints would also be protective for functional endpoints. Otherwise, ecosystem functions should be considered in risk assessment.



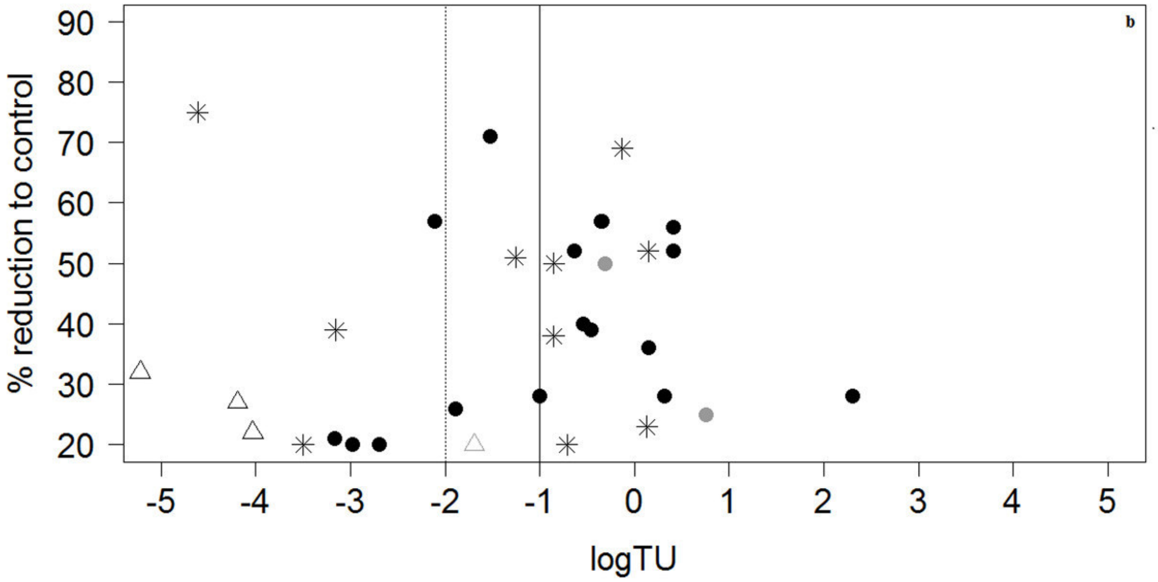


Fig. 2.1 a) Reductions in leaf litter breakdown (filled dots), primary production (asterisk) and community respiration (triangle) in % relative to the control depending on the logTU (based on *D. magna* as standard test organism for invertebrate-related ecosystem functions and *P. subcapitata* for ecosystem functions related to aquatic plants and microorganisms) for heavy metals (40 observations). **b)** Reductions in leaf litter breakdown (filled dots), primary production (asterisk) and community respiration (triangle) in % relative to the control depending on the logTU for organic toxicants (33 observations). The applied UP thresholds of 0.01 (dotted line) and 0.1 (solid line) refer to ecosystem functions provided by invertebrates and microorganisms or aquatic plants, respectively. Grey sampling points indicate organic substances that are no pesticides.

2.4.2 RELATIONSHIP BETWEEN STANDARDIZED CONCENTRATIONS AND EFFECTS ON ECOSYSTEM FUNCTIONS

No concentration-effect relationship could be derived for the three ecosystem functions because the TUs exhibited no explanatory power for the investigated endpoints (linear model for TU and respective ecosystem function: leaf litter breakdown: $r^2=0.04$, $p=0.2$; community respiration: $r^2<0.01$, $p=0.8$; primary production: $r^2=0.05$, $p=0.44$). Consequently, it was not possible to establish a statistical model, which could serve the purpose to derive an effect threshold and subsequently to compare the sensitivity of ecosystem functions. Though effects below 20% were not considered, we expected an increasing effect with an increase in TU. With respect to organic toxicants, this general trend was indicated: Only 2 of 10 observations showed effects ≥ 50% for a TU < 0.01, whereas 11 of 23 observations showed effects ≥ 50% for a TU > 0.01 (Fig. 2.1b). No such tendencies were found for heavy metals (Fig. 2.1a). Two main reasons may explain these results: First, the TU approach used here may have been an unsuitable indicator of ecotoxicity hampering the establishment of a relationship with ecosystem functions. For example, the selected standard test organisms might not be an appropriate benchmark for ecosystem functions (chapter Calculation of toxic units). Second, two substances with the same EC50 may exhibit substantially different slopes with regard to their concentration-effect relationship. Hence, the same nominal TU for two substances may result in distinctly variable effects on organisms, which in turn increases the variation in the relationship between TU and the functional endpoints reviewed in the present study. However, the applied TU approach lead only to minor variation in previous studies e.g. (Liess et al., 2008; Rasmussen et al. 2012; Schäfer et al., 2012b) and two further studies found a strong relationship between TU and ecosystem functions for organic toxicants (Rasmussen et al., 2012; Schäfer et al., 2012b). Moreover, differences in the slopes of concentration-effect relationships should only lead to higher variability but not to biasing complex mixtures, since under- and overestimation would cancel out. We therefore argue that primarily the high variability in biotic and abiotic factors among studies hampered the derivation of a concentration-effect relationship.

With respect to biotic factors, differently composed communities provided the ecosystem functions in each of the studies considered in the present review. Beketov et al. (2008) have shown that such structural differences may influence the sensitivity to toxicants. Moreover, effects from the same exposure concentration on ecosystem functions may vary due to differences in the ability of communities to compensate for toxicant-induced species loss (Cadotte et al., 2011). Finally, the exposure times of the included studies ranged from 1.5 hours to 385 days and given that the sampling was conducted episodically and not continuous, it is very likely that most studies did not measure the largest effect. In other studies partial recovery at the time of sampling may have lead to lower reported effect sizes.

Abiotic factors can also cause high variability in toxicant effects on ecosystem functions. Several studies demonstrated that differences in physicochemical parameters such as pH and temperature can strongly affect bioavailability of toxicants and subsequent effects (e.g. Kashian et al., 2004; Franklin et al., 2000; Fisher, 1991; Lydy et al., 1990). For example, Franklin et al. (2000) found a decline in copper toxicity to green algae with decreasing pH, while Fisher (1991) observed the opposite tendency for pentachlorophenol effects on midge larvae. Furthermore, Lydy et al. (1990) have shown increasing parathion toxicity for midge larvae with raising temperature. Finally, a recent study argued that the abiotic conditions may lead to 1-2 orders of magnitude differences in the sensitivity to toxicants (Liess and Beketov, 2011). Overall, we suggest that differences in the abiotic conditions, biological systems and experimental design among the reviewed studies impede the derivation of a joint concentration-effect relationship and do not allow for a systematic risk assessment.

2.4.3 EXPLAINED VARIABILITY IN ECOSYSTEM FUNCTIONS

The decomposer-detritivore community explained 21% of the variability in leaf litter breakdown among studies (r^2=0.21, F=12.23, $p < 0.01$, BIC = 245.4). The effect was greater when invertebrate detritivores were involved (mean reduction of 58%) than when microorganisms decomposed the organic material alone (mean reduction of 44%). The more pronounced effect in invertebrate mediated leaf decomposition may be attributed to a decline in the functional ability of detritivores (Cadotte et al., 2011; Schäfer et al., 2012b), which play a dominant role in leaf litter breakdown (Peterson and Cummins, 1974; Iversen et al., 1982; Wallace et al., 1982). Detritivores have longer reproduction cycles than microorganisms, which increases the time until recovery. In addition, due to their faster reproduction microorganisms may have acquired a greater tolerance to toxicants (Blanck and Wangberg, 1988). Finally, microorganisms can be assumed to possess greater functional redundancy compared to detritivore communities (Cadotte et al., 2011).

The automatic model building for community respiration and primary production suggested only the inclusion of the type of ecosystem (lotic or lentic) in the final model (community respiration: r^2=0.18, F=2.47, p = 0.14, BIC = 55.1; primary production: r^2=0.18, F=2.58, p=0.13, BIC=85.4). For community respiration the effect was greater in lotic (mean=38%) than in lentic (mean=31%) study systems, whereas primary production showed the opposite tendency (lotic mean=13%, lentic mean=30%). Neither exposure time (community respiration: n=13, p=0.23; primary production: n=14, p=0.73) nor the log TU (community respiration: n=13, p=0.38; primary production: n=14, p=0.62) were significantly different between the ecosystem types for the respective functional endpoints and can therefore not explaining the differences. The results for community respiration are in agreement with a comparison of the sensitivity of organisms in lentic and lotic freshwater ecosystems in the United Kingdom, which indicated a higher sensitivity of lotic organisms (Biggs et al., 2007).

Nevertheless, the variable ecosystem type exhibited no statistical significance (community respiration: p=0.14, primary production: p=0.13) and the null model exhibited only negligibly higher BIC values (community respiration: null model BIC: 55.2 vs. final model BIC: 55.1, primary production: null model BIC: 85.5 vs. final model BIC: 85.4). Hence, these results should be interpreted with caution and may only represent a statistical artifact or be driven by experimental differences between the conditions in the lotic and lentic studies.

2.5 CONCLUSIONS

A safety factor of 100 or 10 of the EC50 for *D. magna* or *P. subcapitata*, respectively, may not be sufficient for pesticides to protect functional endpoints. Since neither estimated toxicity nor other experimental conditions explained variability in the ecosystem functions in our study, working towards method standardization is required if ecosystem functions are to be considered as protection goal in chemical risk assessment. However, it remains open whether protection of structure would also protect function and consequently whether consideration in risk assessment is required.

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- Chapter 3 -

ORGANIC MATTER BREAKDOWN IN STREAMS OF CONTRASTING ANTHROPOGENIC LAND USE

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3.1 ABSTRACT

Streams provide ecosystem services to humans that depend on ecosystem functions, such as organic matter breakdown (OMB). OMB can be affected by land use-related disturbance. We measured OMB in 29 low-order streams in a region of contrasting land use in south-west Germany to quantify land use effects on OMB. We deployed fine and coarse mesh leaf bags in streams of forest, agricultural, vinicultural and urban catchments to determine the microbial and invertebrate-mediated OMB, respectively. Furthermore, we monitored physicochemical, geographical and habitat parameters to explain potential differences in OMB among land use types and sites. Only microbial OMB differed between land use types. Microbial OMB was negatively correlated with pH and invertebrate-mediated OMB was positively correlated with tree cover. Generally, OMB responded to stressor gradients rather than directly to land use. Therefore, the monitoring of specific stressors may be more relevant than land use to detect effects on ecosystem functions, and to extrapolate effects on functions, e.g. in the context of assessing ecosystem services.

3.2 INTRODUCTION

Freshwater ecosystems provide central ecosystem services to humans (Palmer et al., 2004), such as clean drinking water or food (e.g. fishery products: MEA, 2005). Human activities in river basins can seriously impair streams through reach and catchment-wide influences that may result in large scale alterations in stream ecosystem functions (Allan et al., 1997). Ecosystem functions such as primary production and organic matter breakdown (OMB) are pivotal for ecosystem services. OMB represents the most important energy source in terms of organic carbon in the first ten kilometers from the stream source, especially in forested headwaters (Wallace et al., 1997). After the input of organic matter from the riparian vegetation the benthic stream community (i.e. microbial decomposers and invertebrate detritivores) colonize, degrade and then shred the leaf material which provides energy for local and downstream food webs (Webster et al., 1999). This fundamental ecosystem function is impacted by the individual and joint occurrence of stressors such as pollution, anthropogenic nutrient enrichment and hydromorphological changes (e.g. Allan and Castillo, 2007; Paul et al., 2006, Aristi et al., 2012; Piggott et al., 2012).

According to the Millennium Ecosystem Assessment (MEA, 2005), anthropogenic land use is an important stressor for freshwater ecosystems. For instance, in the course of agricultural intensification the riparian vegetation has been increasingly logged, resulting in erosion, reduced input of coarse organic matter and consequently a reduction of available energy (Campbell et al., 1992). Furthermore, several studies reported land use effects on the benthic stream community (e.g. Delong and Brusven, 1998; Urban et al., 2006) or large-scale effects on OMB (e.g. Woodward et al., 2012), though the effects of land use on OMB were often contrasting. As an example for agricultural land use effects, Magbanua et al. (2010) found no significant differences in OMB between conventional, integrated and organic farming, while Piscart et al. (2009) observed a decrease in the OMB rate with increasing agricultural intensity. Similarly, inconsistent results were reported for effects of urbanization (cf. Sponseller and Benfield, 2001 vs. Pascoal et al., 2005). These differences between studies could originate from variable associations of stressors with specific land use types as well as from variable responses to stressors. Moreover, most of the previous studies were conducted on a local scale (i.e. ≤ 5 streams per land use type), considered only one land use type and investigated effects during spring, while the peak of litter fall in regions dominated by deciduous trees occurs in autumn. The local approach, i.e. strong influence of particular environmental variables in a specific catchment, as well as the relatively small sample size (total $n \leq 10$ in most studies) could further explain the inconsistent effects and high variability between the studies. Based on these findings, it is difficult to extrapolate land use effects to larger spatial scales, for example to determine losses of ecosystem functions and services, which has been done on several occasions in ecosystem service assessment (Maes et al., 2012). Extrapolation would require the establishment of a link between land use and ecosystem function on the respective scale, also considering that anthropogenic stressors operate over multiple spatial scales (Allan, 2004). Moreover, regulatory frameworks such as the Water Framework Directive require freshwater management from a catchment perspective, stipulating regional scale studies that include an assessment of stream ecosystem functions (Vighi et al., 2006).

In this study, we aimed to quantify the influence of multiple land use types (i.e. agriculture, viniculture, urbanization) on OMB at a regional scale (i.e. sampling sites located in different catchments) during leaf-fall in autumn. In addition, we intended to identify environmental factors that explain this influence. We hypothesized that OMB differed among land use types and that OMB is higher in anthropogenically-influenced sites due to elevated nutrient discharge.

3.3 MATERIAL AND METHODS

3.3.1 STUDY AREA AND SAMPLING SITES

The study area was located in the south-west of Germany, between the Palatinate forest nature park in the North and West, the River Rhine in the East and the Vosges in the South (Figure B.1 in App. B). To quantify the effect of land use on the OMB, we selected sampling sites in each of eight first-to-third-order streams in forested (F), agricultural (A) and vinicultural (V) areas and in seven streams in urban (U) areas. F sites were considered as relatively pristine, whereas A, V and U sites were considered as subject to different anthropogenic stressors. All streams originated in the Palatinate forest nature park, except for two agricultural streams (cf. App. B, Fig. B.1), and were mainly small, fine substrate-dominated siliceous highland streams. Thus, geology and climate were very similar for the streams and their upstream catchments were relatively pristine. Sampling sites of A, V and U were located within a distance of five km from the edge of the nature park and selected to mainly represent the respective land use type. In addition, the sites were located upstream from drainages or discharges. U sites were chosen downstream from settlements with at least 2500 inhabitants, but upstream from wastewater treatment plants to avoid variability originating from differences in chemical input. The settlements were rather small (between 2500 and 10000 inhabitants) but categorized as urban because the streams were hydromorphologically modified (streambed was channelized). Each site was located in a different catchment and in a different stream, except for three forested sites that were in the same catchment or stream as two urban and one vinicultural site (App. B, Fig. B.1). Given that most streams originated in the Nature Park, the land cover in the stream catchments was mainly forest. Therefore, we based our site selection on the land use within a 100-m wide buffer zone of 3 km length upstream of each sampling site. This buffer zone was selected based on a recent study that found a similar or a slightly stronger ecological response to riparian (buffer zone 100-m wide) compared to catchment-scale land use (Feld, 2013). We assessed land use employing Corine Land Cover (CLC) maps (Büttner and Kosztra, 2007) and created buffers using a geographical information system (QGIS Development Team, 2014). We assigned each site the dominant land use type (> 50% areal cover) within each buffer zone. The assigned land use categories were confirmed during site visits. Overall, we suggest that our site selection process guaranteed that potential effects can be attributed to the selected land use type. Two sites had to be omitted due to drying out (one vinicultural stream) and vandalism (one urban site).

3.3.2 LEAF DEPLOYMENT AND CALCULATION OF BREAKDOWN RATES

The study was conducted in autumn 2012 from September to October. Approximately 3 ± 0.07 g of oven – dried (60°C for 24-h) black alder leaves (*Alnus glutinosa* – collected from a locally common riparian tree species) were placed into coarse polyethylene mesh bags (mesh size: 8mm, bag size: 20 x 20 cm) accessible to microbial decomposers and invertebrate detritivores and into fine cylindrical nylon bags (mesh size: 250µm, cylinder length: 15cm) accessible only to microbial decomposers. Five replicates of each bag type were deployed in each sampling site. Leaf bags were fixed a few centimeters above the stream bottom. After 21 days the leaf bags were recovered from the streams, remaining leaf material was carefully removed from the bags, rinsed to remove mineral particles, oven dried at 60°C (24h), reweighed and averaged for each type of bags (i.e. coarse and fine) for every site. To correct for handling loss, five replicates of each bag type were treated the same way as the others but returned to the laboratory immediately after brief immersion in the stream. To correct for leaching loss the same amount of bags were retrieved after a 24-h stream deployment.

Furthermore, the water temperature was recorded hourly with Synotech HOBO© temperature/data loggers 64K (Hückelhoven, Germany). This allowed us to calculate the sum of degree days ($ddays^{-1}$) for the deployment period of leaves, which was used to standardize the breakdown rate for temperature. The breakdown rate k in a site i was calculated based on the exponential mass loss per ddays (dd^{-1}):

$$k_i = \frac{-\ln\left(\frac{S_i(t)}{S_i(0)}\right)}{\sum_{j=1}^{t} \overline{T}_i(j)}$$

where S is the mass as a function of deployment time t (in days) and T is the mean temperature for a day j. $S_i(t)$ of leaves in fine and coarse mesh bags was corrected for handling and leaching losses. Moreover, $S_i(t)$ of leaves in coarse mesh bags was corrected for microbial breakdown to determine the contribution of invertebrates to breakdown (for details see: Benfield, 2007). Finally, we calculated the proportion (in percentage) of invertebrate-mediated OMB (OMB_{macro}) and microbial OMB (OMB_{micro}) of total OMB to assess the respective contribution to OMB.

3.3.3 ENVIRONMENTAL VARIABLES

To identify the environmental variables that could explain the influence of the selected land use types, we measured the following physicochemical parameters: water temperature, pH, oxygen, electrical conductivity (EC), flow velocity and nutrient concentration (nitrate, nitrite, ammonium, phosphate) by on-site analysis with Macherey-Nagel visocolor® (Düren, Germany) kits. Following a protocol of the EPA (2003), three defined geographical parameters were monitored within a 50-m stream section at each sampling site: mean percent of shading, width of riparian zone in meters (mean of left and right bank) and percent tree cover within the riparian zone (left and right bank maximum). Furthermore, the area of the upstream catchment of each sampling site was calculated using QGIS 2.6.1 (QGIS Development team, 2014). Moreover, we visually recorded stream substrates and microhabitats in the 50-m reach of each sampling site, as detailed in the AQEM/STAR protocol (AQEM, 2002). All measured environmental variables (n = 29) are listed in Table 3.1.

3.3.4 STATISTICAL ANALYSIS

Before analysis, explanatory variables were log- or double square-root transformed in case of strong skewness, which was evaluated based on visual inspection. We examined differences among land use types in each environmental variable and the breakdown rates by parametric ANOVA with F-test in case of homogeneity of variance and normal distribution, followed by Tukey post-hoc test for pairwise differences. Otherwise the non-parametric Kruskal-Wallis test was used followed by Wilcoxon rank sum test. Multiple testing was corrected using Holm correction (Holm, 1979). To visualize how relevant environmental variables (i.e. those variables that significantly differed among land use types after pairwise testing) contributed to variation among the different land use types, we conducted a Principal Component Analysis (PCA) after centering the data to mean and scaling to unit variance (Legendre and Legendre, 2012). Regarding the relationship between environmental variables and OMB, we first checked for collinearity and omitted EC (because it strongly correlated with pH (0.74), oxygen ($r = -0.73$), and nitrate ($r = 0.79$)); stream width and submerse macrophytes (because of strong correlation with catchment area ($r = 0.94$ and 0.74, respectively)). Furthermore, we omitted temperature as the breakdown rates were already standardized for temperature in terms of degree days. Afterwards, we separately conducted manual linear model building to identify variables with highest explanatory power for invertebrate-mediated or microbial OMB. We started with several models each containing a set of explanatory variables and used backward model selection, i.e. eliminated non-significant explanatory variables (t-test for regression coefficient). Additionally, we conducted automatic model building with significant variables from the manual model, with the Bayesian Information Criterion (BIC) as stepwise model selection criterion (Schwarz, 1978). Model checking included homogeneity of variance as well as normal distribution of model residuals and identification of influential observations using residual-leverage plots and Cook's distances. Observations exhibiting a Cook's distance ≥ 0.5 were omitted and the model was refitted. The relative importance of individual variables in the best-fit model was examined with hierarchical partitioning (Chevan and Sutherland, 1991). All calculations and graphics were done using R 3.1.2 (R Development Core Team, 2014).

3.4. RESULTS

3.4.1 ORGANIC MATTER BREAKDOWN AND LAND USE

Even though pairwise post-hoc tests revealed no significant differences in microbial breakdown rates among land use types, the analysis of variance was significant (Kruskal Wallis: $\chi^2 = 8.2$, df = 3, $p = 0.04$; Fig. 3.1a). OMB_{micro} was 25 % lower in F sites compared to U sites. Moreover, it was approximately 20 % and 25 % lower in V and A sites compared to F sites, respectively.

By contrast, OMB_{macro} did not differ significantly among land use types (Kruskal Wallis: $\chi^2 = 0.05$, df = 3, $p = 0.99$; Fig. 3.1b), though it was lower in F sites (e.g. up to 40% lower compared to U sites).

From the significant variables from the manual models, automatic model building identified pH and percent tree cover as governing OMB_{micro} and OMB_{macro}, respectively. pH explained 39 % of the variability (df = 24, $p < 0.0001$) in the OMB_{micro} and exhibited a negative relationship, i.e. the microbial breakdown rate decreased with increasing pH.

Percent tree cover explained 22 % of the variability in OMB_{macro} (df = 26, $p = 0.01$) and the breakdown rate responded positively to an increase in percent tree cover.

Regarding OMB, invertebrates contributed on average as much as microorganisms (i.e. 53 % and 47 %, respectively).

Tal Table 3.1

Median of 29 measured environmental variables (physico-chemical, geographical and microhabitat) for all sampling sites ($n = 29$) by land use types with lower (Q1) and upper (Q3) quartile and information on statistical significances between land use types. Different capital letters indicate significant differences.

Physico-chemical parameter	Median Agriculture	Q1/Q3 Agriculture	Median Forest	Q1/Q3 Forest	Median Urban	Q1/Q3 Urban	Median Vineyard	Q1/Q3 Vineyard
Stream width [m]^a	1.75^{BC}	1.5/2.3	1.8^B	1.7/2.2	4^A	2.9/7.0	1.2^C	1/1.5
Stream depth [m]	0.21	0.16/0.25	0.15	0.1/0.2	0.3	0.25/0.39	0.12	0.11/0.2
Velocity [m^3/s]^b	0.14^{BC}	0.09/0.24	0.29^{AB}	0.25/0.49	0.4^A	0.34/0.74	0.1^C	0.03/0.17
Temperature [°C]^b	12.5^A	12.2/13	11.3^B	11.3/12	12.3^{AB}	11.48/12.9	12.7^A	12.6/12.7
pH^b	8^A	7.9/8.1	7.5^B	7.1/7.6	7.5^B	7.5/7.6	7.9^A	7.8/8
Oxygen [mg/L]^a	9.36^B	8.49/9.58	10.95^A	10.11/10.99	9.4^{AB}	8.97/10.44	8.84^B	8.13/9.6
Conductivity [µS/cm]^b	801^A	550.25/978.5	132^B	117.25/186.75	275^{AB}	161.3/373.3	623^A	304/746
Nitrite [mg/L]^a	0.05^A	0.013/0.085	0.0025^B	0.0025/0.003	0.07^A	0.04/0.09	0.04^A	0.03/0.075
Nitrate [mg/L]^a	20^A	13.75/28.75	3^B	1.75/3	4^{AB}	3/4.75	5^{AB}	4/19
Phosphate [mg/L]	0.2	0.14/0.23	0.1	0.09/0.15	0.3	0.175/0.35	0.35	0.3/0.4
Ammonium [mg/L]	0	0/0.005	0	0/0	0	0/0.05	0	0/0
Minerally substrates (grain size)								
Megalithal (>40 cm)^c	0	0/0	0	0/5	0	0/4	5	3/8
Makrolithal (>20–40 cm)	0	0/1	0	0/1	5	0/8	0	0/5
Mesolithal (>6–20 cm)	3	0/14	8	0/21	5	3/45	10	5/22
Mikrolithal (>2–6 cm)	3	0/21	5	4/11	5	0/5	0	0/8
Akal (>0.2–2 cm)	0	0/0	0	0/5	0	0/0	0	0/3
Psammal (>6 m–2 mm)	20	13/31	48	25/50	35	8/60	35	10/43
Technolithal (artifical substrate)^c	0	0/0	0	0/0	0	0/0	5	3/5
Organic substrates								
Algae	0	0/0	0	0/0	0	0/0	0	0/0
Submerse macrophytes^c	0	0/0	0	0/0	2.5	0/9	0	0/0
Emerse macrophytes	0	0/8	0	0/1	0	0/0	0	0/0
Living parts of terrestrial plants (fine roots, etc.)^c	10	9/16	2.5	0/10	7.5	1/10	15	13/18
Xylal (e.g. woody debris)^a	3^{AB}	0/13	18^A	10/21	5^{AB}	0/10	0^B	0/0
CPOM (i.e. leaf litter)	8	5/15	8	4/10	0	0/5	0	0/8
Organic mud (sludge)^a	10^A	4/15	0^B	0/0	2.5^{AB}	0/9	5^{AB}	0/8
Geographical parameter								
Catchment area [km^2]^a	18.8	14/26	11.7	8/14	98.2	35/244	7.2	5/10
Riparian zone [m]^b	2.5^B	1.5/2.6	6^A	4.8/7.5	2.75^B	2/3	1.5^B	1.4/1.9
Tree cover [max %]	50–80%		50–80%		>80%		50–80%	
Shading [mean % ± SD]	51–75% ± 5%		51–75% ± 5%		51–75% ± 5%		>76% ± 0	

[a] $p < 0.05$ in Kruskal–Wallis test with Wilcoxon rank sum post-hoc test.

[b] $p < 0.05$ in ANOVA with Tukey post-hoc test.

[c] $p < 0.05$ in ANOVA or Kruskal–Wallis test, but no significant pairwise differences.

3.4.2 ENVIRONMENTAL VARIABLES AND LAND USE

The parametric ANOVA and/or Kruskal-Wallis test identified 16 out of 29 environmental variables as significantly different among land use types (see Tab. 3.1 for details on pairwise differences). The first two principal components explained 64% of total variation in the selected environmental variables (Fig. 3.2). The first component separated urban and forested sites from agricultural and vinicultural sites, whereas the second axis primarily separated urban and forested sites. Urban sites typically had larger catchments, wider streams, higher flow velocities and nutrient enrichment, especially in nitrite (and phosphate, not displayed; cf. Tab. 3.1). Sites of forested streams showed higher concentration of oxygen and woody debris (xylal) and wider riparian zones. By contrast, agricultural and vinicultural sites showed the highest pH and EC, highest nutrient enrichment as well as the largest amount of organic mud. Moreover, A and V sites exhibited significantly narrower riparian zones compared to forested sites ($p \leq 0.05$, cf. Tab. 3.1), but a similar cover with riparian trees along the bank (Kruskal Wallis: $\chi^2 = 5.9$, df = 3, p = 0.12, cf. Tab. 3.1).

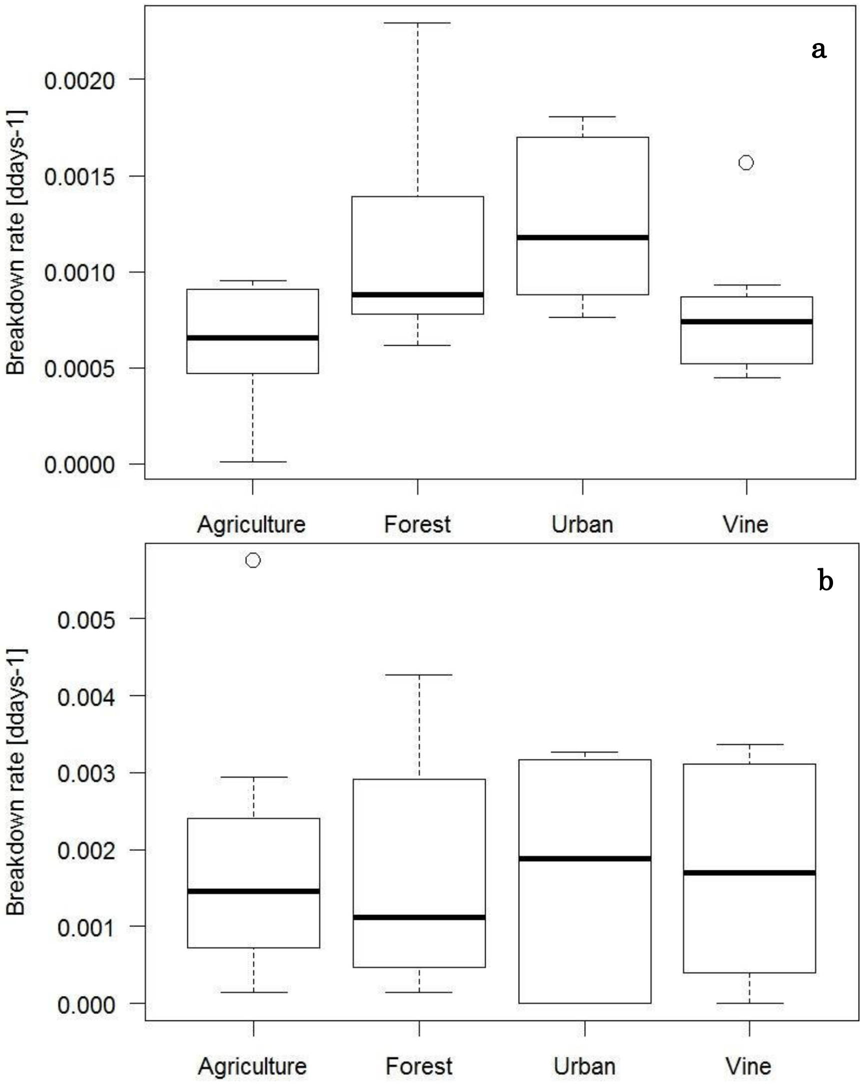

Figure 3.1: Boxplots of **a)** microbial breakdown rates and **b)** invertebrate-mediated breakdown rates of alder leaves among land use types (study period: 21 days, total n = 29).

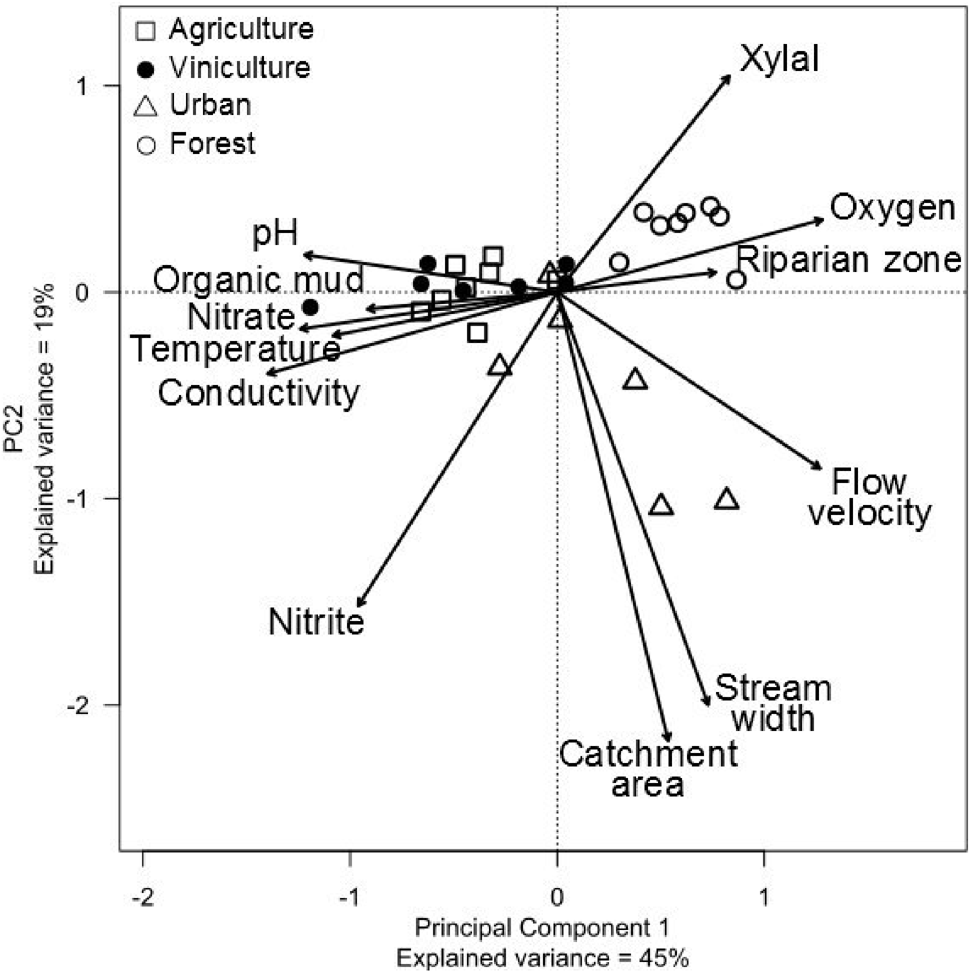

Figure 3.2: Principal component analysis (PCA) biplot for 12 environmental variables that differed significantly among land use types. Variables are plotted as arrows that point in the direction of their maximal variation and the lengths are proportional to their maximal rate of change. The closer the arrows to each other the higher their correlation, and the smaller the Euclidean distance among sampling sites (cf. key) the more similar they are regarding variables (Ramette, 2007).

3.5. DISCUSSION

3.5.1 ORGANIC MATTER BREAKDOWN AND LAND USE

Microbial OMB was significantly affected by land use, whereas OMB_{macro} exhibited no significant effects. The best-fit linear model identified pH as the main influence of OMB_{micro}. In the context of pH as a stressor, most studies investigated the effects of acidification on OMB and found, for instance, a positive relationship between OMB_{micro} and pH (range 4.7 – 7.1; e.g. Dangles and Chauvet, 2003). In our study, the pH values were higher and the gradient relatively short (range 7.0 – 8.3). For a similar pH range (6.5 – 8.2), an increasingly alkaline pH reduced the diversity and density of the fungal community (Casas and Descals, 1997). This may explain the reduced OMB_{micro} at higher pH in our study as fungi are mainly responsible for OMB_{micro} (Hieber and Gessner, 2002). However, a large-scale study on the effects of nutrients on OMB demonstrated that nutrients and OMB can exhibit a hump-shaped relationship and reduce OMB_{micro} for alder leafs when nutrient concentrations exceed approximately 10 mg/L (Woodward et al., 2012).

Indeed, we observed a statistically significantly lower OMB_{micro} for sites with nitrate concentrations exceeding 10 mg/L ($p = 0.002$, Welch two sample t-test, App. B, Fig. B.2). Nevertheless, in contrast to Woodward et al. (2012), OMB_{macro} did not decrease when nitrate concentrations exceeded 10 mg/L ($p = 0.69$, Welch two sample t-test, App. B, Fig. B.3). Moreover, additional stressors such as sedimentation (Niyogi et al., 2003) and toxic agrochemicals (Rasmussen et al., 2012), which can be collinear with pH (Schäfer et al., 2007), may have contributed to a reduction in OMB_{micro}. Although these stressors were not measured, previous studies in streams of our sampling region reported fungicide concentrations (e.g. Bereswill et al., 2012, Fernández et al., 2014) that can affect microorganisms (Dijksterhuis et al., 2011). To sum up, a multiple stressor complex including pH, nutrients, sedimentation and toxic agrochemicals may have influenced the response of OMB_{micro} (Bärlocher, 1992). This interpretation is consistent with the highest OMB_{micro} occurring in urban sites (Fig. 3.1a), where (a) pH was lower than in A and V sites, (b) sedimentation and toxic agrochemicals can be considered minor given the small size of the settlements and (c) nitrate concentrations were below 10 mg/L. Thus, nutrients may have stimulated OMB_{micro} in urban sites, which is in line with Pascoal et al. (2005). Overall, a more mechanistic study would be required to confirm our interpretation.

Concerning OMB_{macro}, percent tree cover exhibited a positive relationship. This may be explained by several factors including (a) an increased supply of allochthonous organic matter (i.e., woody debris and leaves) that lead to an increase of shredder density and (b) a reduction in the input of other stressors such as sediments or toxic agrochemicals through riparian cover. As mentioned above, our hypothesis of higher OMB due to nutrient enrichment has to be rejected for invertebrates. Hence, our study highlights that the interactions of different variables influencing OMB can complicate the detection of clear relationships with individual stressors such as nutrients, especially considering that land use is typically associated with multiple stressors.

OMB_{macro} contributed slightly more than half to the total OMB, which is in agreement with a study attributing at least 50% of total OMB to invertebrates (Hieber and Gessner, 2002). OMB was influenced by gradients in environmental parameters (i.e. in our case, pH and percent tree cover) which were only marginally related to land use. This suggests that the land use categories are an unreliable predictor for potential effects on ecosystem functioning in streams of our study. However, the high variability in OMB within land use categories may have hampered the detection of land-use related differences in OMB on a regional scale. This high variability of OMB may be attributed to a number of factors, such as differences (i) in the influence of co-occurring multiple stressors (Aristi et al., 2012), (ii) in sediment loadings (Niyogi et al., 2003) associated with the sandstone geology of all catchments and (iii) in the biomass or densities of shredders (e.g. Hagen et al., 2006), which may have varied unrelated to land use across the different stream catchments. Furthermore, Peru and Doledec (2010) found that up to a certain level of contamination ecosystem functions can remain stable due to functional redundancy of decomposers. Finally, the spatial proximity of all sampling sites to the nature park may have resulted in the amelioration of land use effects, given that other studies demonstrated the amelioration of effects from toxic agrochemicals for similar distances to undisturbed upstream sections (Schäfer et al., 2012).

In situations with more intense land use (e.g. larger urban area), differences in OMB could be more pronounced when considering that intensive agriculture and urbanization can be associated with distinct stressor profiles, e.g. with non-point and point contamination, respectively. This might enable the detection of a tight link between land-use and ecosystem functions. However, freshwater ecosystems are connected ecosystems where local alterations in the network can propagate downstream. In our study, we intentionally selected a situation with pristine upstream catchments to identify specific effects. For larger catchments with more intense and mixed land use in the upstream catchments, the detection of a clear land use signal may also be hampered.

3.5.2 ENVIRONMENTAL VARIABLES AND LAND USE

The PCA confirmed that F sites in the natural park area were relatively unaffected by human activities. These streams exhibited the widest riparian zones, a high flow velocity, the highest amount of woody debris and the least amount of organic mud, all of which may have contributed to the highest levels of oxygen in these sites. Regarding the environmental characteristics of the three anthropogenic land use types, U sites were the least disturbed (cf. Fig. 3.2). The fact that our U streams were relatively similar to the F streams may be attributed to (i) the selection of sampling sites upstream from wastewater treatment plants or drainages that have been shown to strongly influence the functional integrity of urban streams in other studies (Paul and Meyer, 2001), (ii) the small size of the settlements (2500 to 8000 inhabitants) and (iii) the spatial proximity of U sites to the nature park that promotes recolonisation after disturbances (Sundermann et al., 2011). However, U streams exhibited wider stream widths and higher flow velocities than F streams due to larger catchments and presumably due to hydromorphological changes. Moreover, U and F sites were separated through chemical pollution, i.e. nitrite and phosphate were 26-times and 2.5-times higher in U than in F sites, respectively. Nutrient enrichment, especially with nitrogen and phosphorus, has been well documented in urban and agricultural streams (Pascoal et al., 2005; Paul et al., 2006) and contributed to high EC values, which were also measured in A and in V sites. Furthermore, the reduction in stream flow in agri- and vinicultural streams, together with other factors such as increased sediment input that typically occurs in agricultural streams (Niyogi et al., 2003), may explain the elevated organic mud and oxygen depletion in A and V sites (Carpenter et al., 1998). A and V streams also exhibited the highest temperatures. Although the shading was similar among sites (cf. Tab. 3.1), riparian zones of A and V sites were narrower. This may have resulted in higher solar radiation and in turn higher temperatures (Webb et al., 2008) in A and V streams. Additionally, Poole and Berman (2001) suggested anthropogenically alterations of the flow regime as a source of higher water temperatures. This might also explain the higher temperatures in U sites compared to F sites. Our results confirm that anthropogenic land use such as urbanization or agriculture leaves a fingerprint in the physicochemistry as well as habitat structure.

3.5.3 REGIONAL LAND USE EFFECTS AND EXTRAPOLATION

The Millennium Ecosystem Assessment (MEA, 2005) and recent studies (e.g. Woodward, et al., 2012) have called for a stronger integration of ecosystem functions and services into freshwater management. OMB can be considered a holistic indicator for stream integrity that integrates community and ecosystem-level processes (Gessner and Chauvet, 2002) and can be used complementarily to structural endpoints. We gathered data on OMB of 26 catchments (in total approximately 1,446 km^2) and found that the variation in environmental variables and OMB itself was too high (within as well as among land use categories) to establish a tight relationship with land use on a regional scale. However, such a relationship would be required to allow for extrapolation to other catchments or within the catchments. Thus, land use categories are unsuitable to predict and quantify losses of ecosystem functions and services on regional or larger scales, which is often done for terrestrial ecosystems in ecosystem service assessment (Maes et al., 2012). Nevertheless, OMB responded to gradients of land use-related variables. Such variables could enable extrapolation. However, the influence of individual environmental variables often depends on the environmental context such as co-occurring stressors (Aristi et al., 2012, Clements et al., 2012). This stresses the importance of understanding stressor interactions before a successful extrapolation may be achieved. Finally, even if extrapolation or prediction of effects on ecosystem functions could be achieved, the quantification of ecosystem service losses, which has been called for (Boyd & Banzhaf 2007), may be difficult. This is because of the complex interplay between ecosystem functions and services, where many services can depend on several ecosystem functions (Costanza et al., 1997). Thus, complementing current monitoring efforts by measurements of ecosystem functions such as OMB and primary production (Woodward et al., 2012), which provide energy to the freshwater ecosystem and are consequently pivotal for services such as water purification, may be most promising as an endpoint of interest for human well-being.

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- Chapter 4 -

TAXONOMIC AND FUNCTIONAL DIVERSITY OF STREAM INVERTEBRATES ALONG AN ANTHROPOGENIC STRESS GRADIENT

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4.1 ABSTRACT:

Anthropogenic stress has been identified as main driver of freshwater biodiversity loss. Adverse effects on the biodiversity of freshwater organisms, such as macroinvertebrates, may propagate to associated ecosystem functions, such as organic matter breakdown (OMB). In this context, the functional diversity of communities has been suggested to be a more suitable predictor of changes in ecosystem functions than taxonomic diversity. We sampled macroinvertebrates and determined OMB using leaf bags along a stress gradient in 29 low-order streams to examine the response of taxonomic diversity (TD) and functional diversity (FD) and their relationship with OMB. The taxonomic richness decreased with increasing anthropogenic stress, whereas the Simpson diversity of communities showed no relationship with the gradient. However, the taxonomic richness was not related to OMB, but the Simpson diversity correlated weakly with OMB. The community structure and 26 % of bio-ecological traits were significantly related to the stress gradient, whereas the functional diversity in terms of Rao's quadratic entropy showed no relationship with the gradient. Moreover, neither bio-ecological nor the functional diversity were related to OMB. This may be explained by the functional dominance of crustaceans. Our results show that neither taxonomic nor functional diversity were suitable predictors of ecosystem functioning.

4.2 INTRODUCTION

Freshwater ecosystems are pivotal for ecosystem services on which human societies depend, such as clean drinking water (Palmer et al., 2004). These services rely on ecosystem functions provided by a range of freshwater organisms that are adversely affected by multiple stressors. Overexploitation, water pollution, habitat loss, flow modification and species invasion have been identified as main drivers of freshwater biodiversity loss (Dudgeon et al., 2006). Several of these stressors are, to varying degrees, associated with anthropogenic land use (MEA, 2005), such as (i) agriculture, which is often associated with water pollution and flow modification or (ii) resource extraction, which is often associated with water pollution. Water pollution in the form of elevated salinity or inputs of pesticides has been shown to reduce the taxonomic diversity (TD) of lotic communities, respectively (Cañedo-Argüelles et al., 2013; Beketov et al., 2013). Land-use related stressors may also affect the functional diversity (FD). Elevated nutrients and pollution with metals and PAHs (Polycyclic Aromatic Hydrocarbons) from sediments as well as alternating flow conditions in urban streams caused shifts in the functional composition of invertebrate communities, resulting in reduced FD (Fanny et al., 2013; Feio et al., 2015; Konrad and Booth, 2005). Overall, the effects of stressors on communities can translate to changes in TD and FD, given that the loss of species can result in the loss of functional traits (Chapin et al., 2000) or that, conversely, environmental filtering for specific traits (i.e. Stressor-tolerance) can control species colonization in a community (Weiher et al., 2011). Changes in the diversity of communities can propagate to ecosystem functioning as both are linked (Naeem and Wright, 2003) through mechanisms such as complementarity, niche partitioning or functional redundancy (Cardinale, 2011; Cardinale et al., 2011; Ebeling et al., 2014). Although the loss in TD has been associated with impairment of ecosystem functions such as nutrient cycling (McIntyre et al., 2007) or organic matter breakdown (McKie et al., 2008), the direction and magnitude of changes in ecosystem functions depends rather on the functional traits of individuals, populations and communities (Cadotte et al., 2011; Tolkkinen et al., 2013). For instance, species loss can be indifferent for ecosystem functions in the case of functional redundancy, i.e. when other species in the community can compensate for the loss of species (Fetzer et al., 2015). However, the loss of species with key functional roles most likely results in a reduction in ecosystem functions

(Cardinale et al., 2006). Although several studies described effects of changes in the FD on ecosystem functions (e.g. Díaz and Cabido, 2001; Flynn et al., 2009; Frainer and McKie, 2015), freshwater ecosystems are still largely assessed using taxonomic indicators (e.g. EPT richness, saprobic index or RIVPACS), which do not allow for a direct evaluation of the status of ecosystem functions. However, organism traits, which form the basis of FD, allow for the connection to ecosystem functions (Cadotte et al., 2011) and often respond rapidly and predictably to multiple stressors (Dolédec et al., 2006; Doledec and Statzner, 2010; Mouillot et al., 2013). Thus, FD-metrics may be better indicators of changes in ecosystem functions than TD- metrics (Cadotte et al., 2011). Yet, few studies have compared TD- and FD-metrics of freshwater organisms in the context of anthropogenic stressors (e.g. Dolédec et al., 2006; Dolédec et al., 2011), especially in the context of stressor effects on ecosystem functions, such as organic matter breakdown (OMB; e.g. Frainer and McKie, 2015; Tolkkinen et al., 2015). OMB represents the most important energy source in forested headwaters and subsequent food webs (Wallace et al., 1997; Webster, 2007). Leaf material from the riparian vegetation is decomposed by microbial decomposers and invertebrate detritivores (Graça et al., 2001; Hieber and Gessner, 2002). Accrued fine particular organic material and gained biomass also subsidizes downstream sections, which emphasizes the importance of OMB (Webster, 2007).

We investigated the response of TD and FD of invertebrate communities to anthropogenic stressors along a land-use gradient and examined whether both metrics would be predictive for the rate of organic matter breakdown. We hypothesized (i) changes in the taxonomic and functional (trait) structure of communities along the land-use gradient due to different adaptations of species to environmental conditions (Jackson and Sax, 2010) and due to environmental filtering for specific traits and related trait combinations (Cadotte, 2009; Verberk et al., 2008), respectively. Accordingly, we hypothesized that (ii) land-use related stressors reduce both TD and FD because they are inherently linked, though not necessarily linearly (Cadotte et al., 2011; Woodward et al., 2015; Schriever et al., 2015). This led to the assumption that (iii) OMB would be reduced through the loss of taxa with OMB-relevant trait modalities. Finally, we expected (iv) FD to be a better predictor of OMB than TD (Wooster et al., 2012; Cadotte et al., 2011).

4.3 METHODS

4.3.1 STUDY AREA AND ENVIRONMENTAL VARIABLES

The sampling sites were located in the south-west of Germany between the Palatinate Forest Nature Park in the North and West, the River Rhine in the East and the Vosges in the South (App. C, Tab. C.1). Twenty-nine sites in low-order streams were selected based on the dominant land-use (>50% areal cover, i.e. forest, agriculture, viniculture and urban areas) within a 100-wide riparian buffer zone of 3 km length upstream (for more details refer to (Voß et al., 2015)). All streams originate in the Palatinate Forest or in Northern Vosges and the studied stream reaches were located within 1 to 5 km from the border of the nature park (except the forested sites), which means that the catchment land use was largely forest and the stream reaches were primarily at risk of potential effects by the anthropogenic land use in the riparian upstream zone. The following physicochemical variables were measured during September and October 2012: flow velocity, oxygen, pH, electrical conductivity (EC), water temperature and four nutrients (nitrate, nitrite, ammonium, phosphate) by on-site analysis with Macherey-Nagel visocolor® (Düren, Germany) kits. Moreover, we recorded habitat-structural variables, i.e. stream substrates and microhabitats at each sampling site within a 50-m stream section, as described in the AQEM/STAR protocol (AQEM, 2002) as well as the percentage of shading, total riparian zone and percentage of tree cover within the riparian zone (left bank and right bank maximum), as described by a protocol of the EPA (EPA, 2003). See supplementary information for the complete list of measured environmental variables (App. C, Tab. C.2). Based on findings by Voß et al. (2015), the measured environmental variables were stronger predictors of OMB than land-use. Thus, in this article anthropogenic stress was rather represented by a land-use related environmental gradient established by using a Principal Component Analysis (for details refer to 2.6 Statistical Analysis) than by land-use categories.

4.3.2 INVERTEBRATE SAMPLING

Invertebrate sampling was conducted in concert with the sampling of environmental variables. Invertebrate sampling was done following the standardized AQEM multi-habitat sampling method (Barbour et al., 1999; AQEM, 2002), which entails sampling of the major habitats proportionally to their presence within a sampled reach. Invertebrates were taken by kick sampling and were collected manually from stones, dead wood or plants. They were identified to species or genus level (exception: e.g. larvae of Chironomidae) using a microscope (Olympus SZX9, Tokio, Japan) and identification keys (Brohmer et al., 2000; Bährmann, 2011). In total 66 invertebrate taxa were identified (App. C, Tab. C.3).

4.3.3 TAXONOMIC DIVERSITY

To quantify effects by changing environmental variables on the invertebrate community we calculated two TD metrics: i) total taxonomic richness (TTR, i.e. number of different taxa found) and ii) the Simpson diversity (SD) of invertebrate taxa. Simpson diversity for each site *k* was calculated as:

$$D_k = \sum_{i=1}^{S_k} p_{ik}^2$$

with S_k, the taxonomic richness of site *k* and p_{ik} the relative abundance of the i^th^ taxa in site *k*. We selected Simpson diversity because we expected that dominant species could be more important (Morris et al., 2014). Furthermore, we used $1/D_k$ instead of $1\text{-}D_k$ to correct for bias encountered for high richness (Peru and Dolédec, 2010). Additionally, we used the reciprocal index as its increase indicates an increase in diversity and consequently simplify interpretation (Magurran, 1988).

4.3.4 FUNCTIONAL DIVERSITY

The FD was determined by combining the relative abundance of the collected invertebrate taxa with ten ecological (i.e. Transversal distribution, Longitudinal distribution, Altitude, Preferences for: Saprobity, Substrate, Current velocity, Trophic status, Salinity, Temperature and pH) and eleven biological traits (i.e. Maximum potential body size, Life cycle duration, Potential number of cycles per year, Aquatic stages, Reproduction, Dispersal, Resistance forms, Respiration, Locomotion and substrate relation, Food and Feeding habits) obtained from the database of Tachet (Usseglio-Polatera et al., 2000). Each trait consists of different modalities (in total 113; cf.: (Usseglio-Polatera et al., 2000)) for which the affinity of a taxon is given. Affinities range from zero (no affinity) to three (maximum affinity) and this information was scaled to the relative affinity of each modality per trait within the community. For the 21 traits we calculated the FD by using „Rao's quadratic entropy" (RQE, cf.: (Peru and Dolédec, 2010)) as follows:

$$\mathrm{RQE}_k = \sum_{i,j=1}^{S_k} p_{ik} \times p_{jk} \left[d_{ij}\right]^2$$

with S_k being the taxonomic richness of site k, and p_{ik} and p_{jk} being the relative abundance of the ith and jth taxa in site k, respectively. $[d_{ij}]$ is the dissimilarity between taxa i and j. The dissimilarity between taxa was calculated as Euclidean distance of PCA-scores for each trait, separately (for more details refer to Peru and Dolédec, 2010). Additionally, the RQE over all 21 traits was calculated. RQE was selected as a measure of FD because it is directly comparable with the SD and it is assumed to be highly accurate regarding functional divergence measurements (Woodward et al., 2015). Furthermore, trait information was assigned to family-level for 16 taxa (out of 66 taxa), to genus-level for 39 taxa and to species-level for 11 taxa. For taxa that could only be identified to family-level, the mean affinities per trait were calculated from all available genera of the respective families (9 out of 16). Moreover, for *Cordulegaster sp.*, *Gammarus sp.*, *Philopotamus sp.* and *Rhyacophila sp.* trait information were only available at genus-level although we identified to species-level. Thus, the related species were assigned the same genus-level information. Consequently, FDs were calculated for 53 taxa instead of 66 taxa found.

4.3.5 DETERMINATION OF ORGANIC MATTER BREAKDOWN

During the main period of litter fall, in September and October 2012 together with the sampling of environmental variables and the invertebrate community, coarse- and fine-mesh leaf bags were deployed at each sampling site (n = 29). Approximately 3 ± 0.07 g of oven – dried (60°C for 24-h) black alder leaves (*Alnus glutinosa* – collected from a locally common riparian tree species) were placed into coarse polyethylene mesh bags (mesh size: 8mm, bag size: 20 x 20cm) accessible to microbial decomposers and invertebrate detritivores and into fine cylindrical nylon bags (mesh size: 250μm, cylinder length: 15cm) accessible only to microbial decomposers. Five replicates of each bag type were deployed in each sampling site and recovered from the streams after 21 days. Furthermore, the water temperature was recorded hourly with Synotech HOBO© temperature/data loggers 64K (Hückelhoven, Germany). This allowed us to calculate the sum of degree days (ddays^{-1}) for the deployment period of leaves, which was used to standardize the breakdown rate for temperature. The breakdown rate k in a site i was calculated based on the exponential mass loss per ddays (dd^{-1}) as follows:

$$k_i = \frac{-\ln\left(\frac{S_i(t)}{S_i(0)}\right)}{\sum_{j=1}^{t} \overline{T_i}(j)}$$

where S is the mass as a function of deployment time t (in days) and T is the mean temperature for a day j. $S_i(t)$ of leaves in fine and coarse mesh bags was corrected for handling and leaching losses. Moreover, $S_i(t)$ of leaves in coarse mesh bags were corrected for microbial breakdown to determine the contribution of invertebrates to breakdown (for details see: Benfield, 2007). Hereafter, we refer to invertebrate-mediated organic matter breakdown as OMB.

4.3.6 STATISTICAL ANALYSIS

Before analysis, environmental variables were log- or double squareroot transformed in case of strong skewness. Given collinearity as indicated by a strong correlation with pH ($r = 0.74$), oxygen ($r = -0.73$), and nitrate ($r=0.79$), electric conductivity was omitted from the analysis. In total, a set of 27 environmental variables was included in our analysis (App. C, Tab. C.2) and centered to mean and scaled to unit variance (Legendre and Legendre, 2012). Given the low n:p ratio (observations to variables), we established environmental gradients using principal components analysis (PCA; based on R packages *vegan* (Oksanen et al., 2015) and *BiodiversityR* (Kindt and Coe, 2005)). The first four principal components (PC1 to 4) were meaningful following the broken stick criterion and together explained 52% of the variability among sampling sites (PC1 = 21%, PC2 = 13%, PC3 and 4 both 9%, App. C, Tab. C.4). PC1 was the only axis that unequivocally represented a gradient of increasing anthropogenic stress with for example: decreasing oxygen levels and flow velocities, and increasing pH, temperatures and nitrate levels. Hereafter, we refer to PC1 as stressor gradient. Moreover, PC1 was the only axis that showed significant relationships with all biotic endpoints in later analyses (e.g. Redundancy Analysis of taxonomic and functional diversities and of aquatic communities) and hence PC2 to 4 were not further considered. The relationship between diversity metrics and both OMB, and the stressor gradient was examined using Pearson correlation. Regarding the relationship between FD and OMB, only two OMB-relevant traits (i.e. Food and Feeding habit) were considered. The explained variance (r^2) was used to assess whether TD or FD was a better predictor of OMB. Multiple testing was corrected using the correction by (Benjamini and Hochberg, 1995). Redundancy analyses (RDA) were conducted to detect stressor effects on the taxonomic and trait structure of invertebrate communities. RDA on the 113 trait modalities were conducted on the relative abundance of each trait modality per sampling site. Species data were Hellinger-transformed prior to RDA to circumvent the problems associated with using the Euclidean distance for ecological data (Legendre and Gallagher, 2001). Significance of RDA axes was tested with an ANOVA-like permutation test based on a pseudo-F statistic (Legendre et al., 2011). All calculations and graphics were done using R 3.2.2 (R Development Core Team, 2015). Computer code and raw data are provided to allow for reproducibility of the analysis (Supplementary data computer code).

4.4 RESULTS

4.4.1 CHANGES IN THE COMMUNITY AND TRAIT STRUCTURE ALONG THE STRESSOR GRADIENT

The invertebrate communities changed along the stressor gradient concerning their taxonomic and their trait structure (Fig. 4.1). The gradient explained 8% and 14 % of total variance in the taxonomic structure ($F = 2.2$, $p = 0.03$) and trait structure ($F = 4.4$, $p = 0.001$), respectively. Taxa, such as *Gammarus roeseli* or *Radix ovata* occurred exclusively in sites with higher anthropogenic stress (e.g. high nutrient levels and pH) whereas *Gammarus fossarum* and *Baetis spec.* occurred more frequently and *Protonemura spec.* or *Sericostoma personatum/flavicorne* exclusively in sites of lower anthropogenic stress (e.g. low nutrient levels but high oxygen saturation and fast flow velocities), respectively (Fig. 4.1a).

Regarding the trait structure, most traits ordered point-symmetrically along the gradient, e.g.: communities preferring lower nutrient levels (i.e. oligotrophic, xeno- and oligosaprobic conditions) and higher nutrient levels (eutrophic,α-meso- and polysaprobic conditions) at the opposite ends of the gradient (Fig. 4.1b). Trait modalities most relevant for OMB (i.e. feeding on *dead plants (>1mm)* and being a *shredder*) showed no clear signal towards low or high anthropogenic stress (Fig. 4.1c).

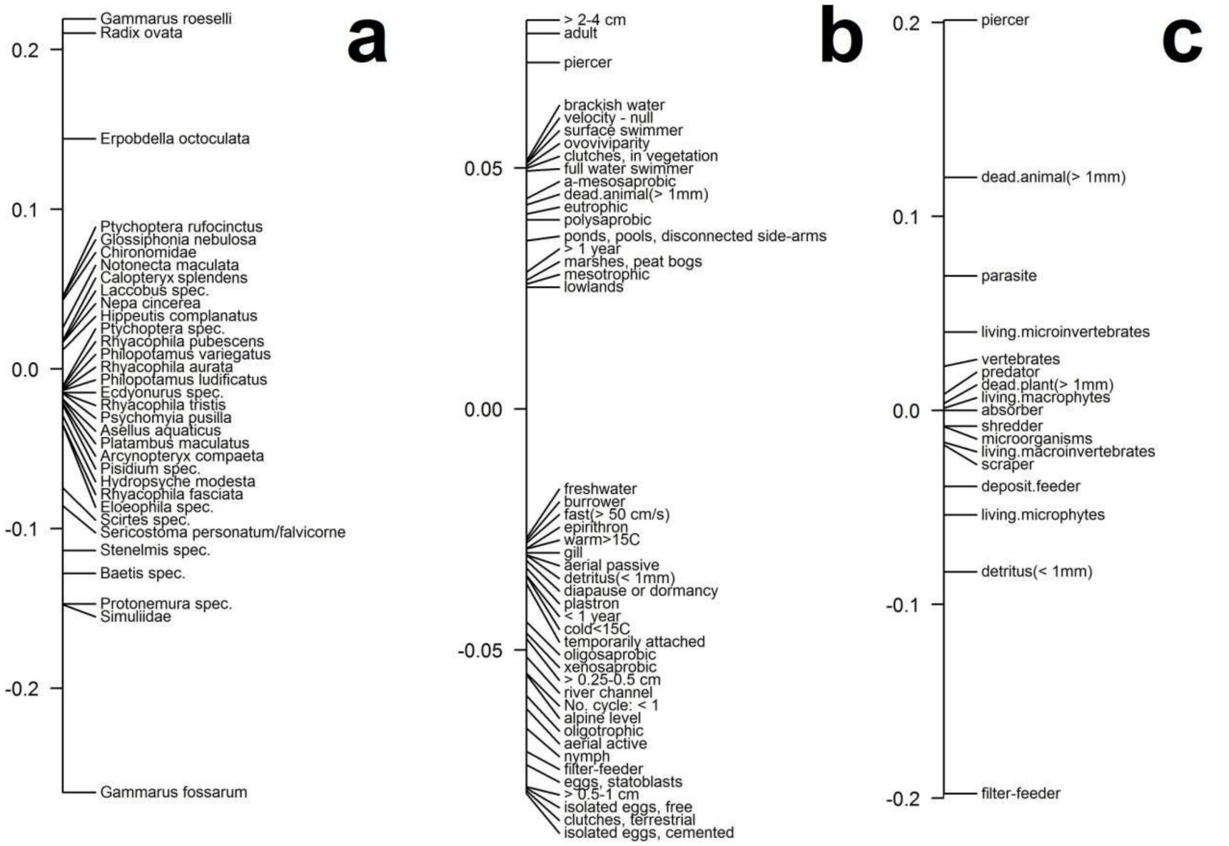

Fig. 4.1: Three graphs each displaying the first RDA-axis (i.e. stressor gradient) along which **a)** invertebrate taxa; **b)** trait modalities and **c)** modalities of OMB-relevant traits (i.e. Food and feeding habits) are listed. For better visibility only (a) taxa and (b) trait modalities with coefficients > 0.01 or > 0.025 were plotted, respectively.

4.4.2 RELATIONSHIP BETWEEN THE STRESSOR GRADIENT AND BOTH TD AND FD

Regarding the TD, the TTR decreased along the stress gradient ($r = -0.53$, $p = 0.009$, Fig. 4.2a), while the SD was not related to the gradient ($p = 0.6$).

Regarding the FD, neither the RQEs calculated for the individual traits (as the FD measure) nor the overall RQE (across all traits) were related to the stressor gradient (App. C, Tab. C.5). However, 30 out of 113 trait modalities were correlated with the stressor gradient (App. C, Tab. C.6). For instance, the relative abundance of taxa preferring xenosaprobic conditions decreased ($r = -0.72$, $p < 0.01$) whereas taxa preferring α-mesosaprobic conditions increased ($r = 0.67$, $p < 0.01$) within communities along the gradient. This reflects the point-symmetrical order along the gradient as found in the trait structure. However, OMB-relevant trait modalities showed no relation with the stressor gradient (App. C, Tab. C.6).

4.4.3 RELATIONSHIP BETWEEN OMB AND BOTH TD AND FD

Neither TTR ($r = 0.02$, $p = 0.9$), the two RQEs of OMB-relevant traits (i.e. Food and Feeding habit), their modalities (App. C, Tab. C.7), nor the overall RQE ($r = -0.2$, $p = 0.29$) were related to OMB. SD showed a weak negative relationship with OMB, though not significant ($r = -0.41$, $p = 0.06$), i.e. decreasing breakdown rate with increasing SD (Fig. 4.2b).

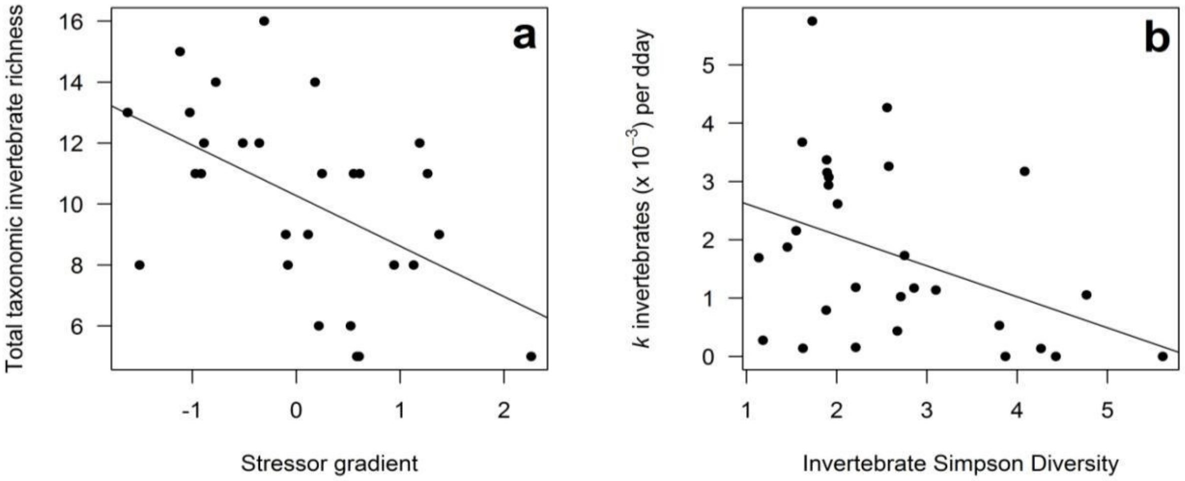

Fig.4.2: Correlations between **a)** the stressor gradient and the total taxonomic richness of 29 invertebrate communities and **b)** the Simpson diversity for 29 invertebrate communities and their respective breakdown rate (*k*). Regression lines are added to visualize the negative correlation.

4.5 DISCUSSION

4.5.1 CHANGES IN COMMUNITY AND TRAIT STRUCTURE ALONG THE STRESSOR GRADIENT

Freshwater species are vulnerable to changing environmental conditions, especially human-driven changes, where vulnerability varies across (and within) taxa (Strayer and Dudgeon, 2010). For example, we found that *Sericostoma spec.* or *Protonemura spec.* and *Erpobdella spec.* or *Radix spec.* occurred at different ends of the stress gradient, which may be explained by environmental filtering for specific traits (Weiher et al., 2011; e.g. sensitivity towards nutrient enrichment). This shift in the taxonomic structure was associated with a functional change in terms of trait modalities (e.g. taxa preferring xenosaprobic conditions decreased whereas taxa preferring α-mesosaprobic conditions increased in communities along the gradient) and the functional structure of communities, which is in agreement with our first hypothesis. However, the low percentage of explained variance of both the taxonomic and the trait structure could result from considering only one environmental gradient (i.e. our stress gradient) and from other factors structuring invertebrate communities, such as interspecific relationships, the spatial context and stochasticity (Loreau et al., 2003; Kraft et al., 2015). For instance, differences in the competitive ability of community members are known to shape communities through the exclusion of the less competitive species (Malmqvist, 2002). In addition, species occurrence in a community can be the result of immigration from neighboring populations (i.e. mass effects) or of random migration, colonization and extinction dynamics (i.e. neutral model; Leibold et al., 2004). Our results are in accordance with other studies investigating land-use related stressors. For instance, the proportions of urban areas within the catchment or hydromorphological changes were identified to alter the taxonomic structure of invertebrate communities, respectively (Aschonitis et al., 2016; Feld and Hering, 2007). Furthermore, a functional homogenization was found in communities due to human-induced nutrient enrichment in Portuguese streams (Feio et al., 2015).

4.5.2 RELATIONSHIP BETWEEN THE STRESSOR GRADIENT AND BOTH TAXONOMIC AND FUNCTIONAL DIVERSITY

The TTR decreased along the stress gradient, indicating the exclusion of sensitive taxa, such as *Sericostoma spec.* and *Protonemura spec.*, from sites with higher anthropogenic stress without compensation by tolerant taxa. In contrast to TTR, the SD of communities was not related to the gradient. This suggests that variables underlying the stress gradient did not cause differences in the SD of communities. Nevertheless, the abundance of gammarids was significantly related to SD (App. C, Fig. C.1a). This can be best explained by SD being a measure of dominance that is heavily weighted towards the most abundant taxa (gammarids in our study, see App. C, Fig. C.2) and less sensitive to changes in rare species (Magurran, 2004). In this context, we hypothesized a reduced TD due to increasing anthropogenic stress. For TTR, this hypothesis can be confirmed whereas for SD it is rejected.

Regarding the FD, metrics such as functional guilds or organism traits were suggested as indicators for assessing the integrity of ecosystem functions or to complement current ecosystem assessment (Feld and Hering, 2007; Vandewalle et al., 2010; Cadotte et al., 2011). Based on these studies, we hypothesized a decline in FD due to increasing anthropogenic stress. Indeed, more than 25% of trait modalities were significantly related to anthropogenic stress, though partly owed to contrasting relationships of trait modalities within a trait (cf. App. C, Tab. C.6). For instance, the relative abundance of taxa preferring xenosaprobic conditions decreased whereas taxa preferring α-mesosaprobic conditions increased along the gradient. This indicates environmental filtering for different traits at the opposite ends of the gradient as underlying mechanism structuring communities. We found no changes in RQEs along the gradient (i.e. the measure of FDs for each trait) indicating no or low functional divergence within communities along the gradient (Woodward et al., 2015). However, potential changes may have been masked by the resolution of the trait information used in this study (Tachet database (Usseglio-Polatera et al., 2000)), potentially ignoring trait differences on the species-level (Bêche and Statzner, 2009). For example, we found a shift in gammarid species along the gradient, indicating different habitat demands, and this shift was not reflected in the trait values as all species were assigned to the same traits. In contrast to studies in vegetation ecology where traits are often directly measured (e.g. Díaz and Cabido, 2001), freshwater invertebrate traits are often assigned from databases resulting in the ignorance of intraspecific variability and adaptations. This might hamper the detection of responses to anthropogenic stress (but see Shipley et al., 2016). Moreover, another methodical artifact could result from information loss associated with calculating a single trait diversity value out of multiple trait modalities (Doledec and Statzner, 2010), of which several individual traits responded to the stress gradient. Furthermore, the length of the stress gradient may have been too short to allow for the detection of potential effects.

Overall, our study shows that despite several functional changes in the communities the FD over several trait modalities remained similar. Although, FD might be an accurate measure of divergence it was not a suitable indicator of gradient-related changes in our study. Thus, focusing on multi-metric indices such as the I_2M_2 that incorporates taxonomic and functional metrics (Mondy et al., 2012) might be preferable when assessing impacts of various stressors on biodiversity.

4.5.3 RELATIONSHIPS WITH OMB

We hypothesized that OMB would be reduced through the loss of taxa with OMB-relevant trait modalities (i.e. feeding on *dead plants (> 1mm)* and *shredding*). Since we did not detect a loss in taxa and their respective OMB-relevant trait modalities, we could not evaluate this hypothesis. However, generally we found no relationship between OMB and TTR, OMB-relevant RQEs and their trait modalities. This may, as for TD, reflect the dominance of gammarids in our study region (App. C, Fig. C.2). Their consistently high number in all communities could have masked potential effects of changes in overall diversity metrics and kept OMB stable. Gammarid dominance might be a result of season, since we sampled invertebrates in autumn in concert with the main litter fall where preceding emergence could have led to more homogeneous communities. Additionally, a lack of habitat complexity could explain the absence of a relationship between OMB and OMB-relevant trait modalities. In this context, (Frainer et al., 2014) found that decomposer traits were related to the most complex habitats, which in turn had the highest rates of OMB. Indeed, our sampling sites were similar regarding measured microhabitats given dominating sandstone geology in our study area (cf. Voß et al., 2015). However, we found a negative, albeit weak, relationship between SD and OMB, i.e. a higher diversity was associated with a lower OMB. Communities with low SD showed highest abundance of gammarids, which was again positively correlated with OMB (cf. App. C, Fig. C.1b), reinforcing their compensating effect on OMB already mentioned and again indicating their functional dominance (Creed et al., 2009; Schwartz et al., 2000; Tolkkinen et al., 2013).

4.6 CONCLUSIONS

For our study, we reject the hypothesis that FD is a better predictor of OMB than TD. Hence, when understanding anthropogenic impacts on the linkage between biodiversity and ecosystem functioning is of main interest, focusing on metrics that are tailored to the respective ecosystem function might be better predictors. For OMB, this could be, for example, metrics that are restricted to the shredder community and that also consider the biomass of shredder species weighted by their metabolic rate. Moreover, when the aim is to assess impacts of various stressors on biodiversity, recently developed indices such as the I_2M_2 that incorporates taxonomic and functional metrics (Mondy et al., 2012) could perform better than FD alone.

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- Chapter 5 -

GENERAL DISCUSSION

Anthropogenic land use and associated stressors threaten freshwater ecosystems and the benefits they provide to human society. This is because the ecosystem functions that regulate these services are adversely affected by anthropogenic land use and associated stressors. This thesis focused on effects by land use in general and in detail by stressors (in terms of heavy metals, pesticides and an established stressor gradient) associated with land use on the fundamental ecosystem function organic matter breakdown. Since the relationships between anthropogenic stressors and ecosystem functions are highly complex this thesis contributes to a better understanding of this relationship. Moreover, it represents a first step towards the preservation of freshwater-related services by identifying the effects of specific stressors on freshwater ecosystem functioning.

5.1 EFFECTS OF ANTHROPOGENIC TOXICANTS ON ECOSYSTEM FUNCTIONS

The 46 studies reviewed in this thesis revealed reductions in organic matter breakdown (OMB), primary production (PP) and respiration (RE) due to anthropogenic toxicants, including pharmaceuticals, heavy metals and pesticides (chapter 2). While reductions in ecosystem functions were not related to heavy metal toxicity, reductions caused by organic toxicants tended to increase with toxicity. However, for any of the two toxicant groups distinct concentration-effect relationships could be established, as the respective toxicities exhibited no explanatory power in statistical models. This lack of a relationship between stressor toxicity and ecosystem functioning most likely resulted from differences in experimental designs among studies (e.g. varying abiotic conditions or biological systems). This can be supported by a meta-analysis investigating heavy metal effects on OMB that calculated a percentage of total variation between studies of 83 % due to high variability in environmental conditions and methodological approaches (Ferreira et al. 2016). Additionally, it has been shown that the response to toxicants can differ depending on community composition (Beketov et al. 2008) or on other mechanisms on which B-EF relationships are based, such as functional redundancy (Cadotte et al. 2011). Similar results can be found for marine ecosystem functions, where effects of toxicants (including metals or herbicides) varied according to the functional or taxonomic groups studied (Johnston et al. 2015). Especially for heavy metals, another explanation can be the high dependence of their toxicities on bioavailable metal concentration and speciation or on other environmental factors influencing bioavailability, (Malaj et al. 2012, Ferreira et al. 2016) since more than half of the heavy metal studies were conducted in the field. Finally, the approach of using TUs as indicators of ecotoxicity could have been unsuitable given that selected standard test organisms might not be suitable benchmarks for ecosystem functions (for details refer to section 2.3.4). On the other hand, the TU approach has already proved its potential to detect changes in functional endpoints associated with organic toxicants (e.g. (Fernández et al. 2015, Münze et al. 2015)) and is also a well-established concept regarding heavy metal and pesticide toxicity on stream organisms (Playle 2004, Schäfer et al. 2012).

Although no concentration-effect relationship could be established, the considered toxicants affected ecosystem functions. According to the UP of the EU for the authorization of pesticides, concentrations below thresholds of 0.01 and 0.1 (corresponding to TU of 0.01 and 0.1; for details see section 2.3) are assumed to cause no or no unacceptable adverse effects on freshwater organisms. As structural changes can propagate to ecosystem functions (Covich et al. 2004, Sutherland et al. 2009) we adopted UP thresholds of structural endpoints for functional endpoints (such as OMB, PP and RE) expecting that ecosystem functions will be unaffected by toxicant concentrations below these thresholds. However, we found effects of organic toxicants, particularly pesticides, on all three ecosystem functions at concentrations up to 1000-fold below the respective UP thresholds. We also found notable reductions in ecosystem functions due to heavy metals at similarly low trace levels as organic toxicants, albeit heavy metal effects were not related to regulatory thresholds (for details refer to section 2.3.4). Hence, our results suggest that OMB, PP and RE can be adversely affected at pesticide concentrations corresponding to TUs of 0.01 and 0.1 and below. Since community data were not available for most of the studies considered in the review, we could not assess whether structural changes below the UP thresholds contributed or were responsible for observed functional changes. Hence, for pesticides it remains open, whether a protective threshold for structural endpoints would also be protective for functional endpoints. However, recent studies revealed adverse effects by pesticides on stream communities (at TU ranges similar to those described above; Fernández et al. 2015, Münze et al. 2015) and on terrestrial invertebrates or insect pollinators at environmental relevant concentrations (Chagnon et al. 2015). In both cases, authors interpreted reductions in ecosystem functioning as a result of community changes.

Against the background of this B-EF relationship, two meta-analyses become alarmingly important regarding the relevance of organic toxicants in threatening stream organisms and potentially ecosystem functions. The first revealed that in 14% and 42 % of investigated sites organic chemicals exerted acute lethal and chronic long-term risk, respectively, on sensitive aquatic organisms (fish, invertebrate or algae species) on a continental scale (Malaj et al. 2014). On a global scale, the second meta-analysis revealed that agriculturally applied insecticide concentrations actually quantified in surface waters exceeded their respective regulatory threshold levels in more than 50 % of the cases (Stehle and Schulz 2015). This illustrates how severe species are threatened (potentially and actually) by anthropogenic pollutants (in this case organic chemicals). When assuming that mean species loss affect ecosystem functioning these two studies exemplify an enormous risk to ecosystem functions. Indeed, a review found a consistent pattern for four different trophic groups (producer, herbivores, detritivores and predators) and two main ecosystem types (aquatic and terrestrial) where species loss on average influence ecosystem functioning with the magnitude of influence depending on the identity of the lost species (Cardinale et al. 2006). Thus, when management plans do not adapt to the complexity of natural ecosystems by implementing trait-based ecological risk assessment (Malaj et al. 2012) or integrating (multiple) ecosystem functions as well as considering large spatial and temporal scale to bioassessment (Woodward et al. 2012) prospects for freshwater integrity and its resilience to disturbances might be poor. However, in the next section I focus on one mechanism (underlying B-EF relationships) that has the potential to maintain ecosystem functions under anthropogenic stress and thus can counteract the propagation of threats on organisms to ecosystem functioning.

5.2 EFFECTS OF LAND USE ON ORGANIC MATTER BREAKDOWN

We investigated effects of anthropogenic land use (agriculture, viniculture and urbanization) and associated stressors on OMB.

We found significant differences in microbial breakdown (OMB_{micro}) rates among land use types, with highest breakdown rates in urban sites. This can be best explained by the stimulating effect of elevated nutrient levels in urban streams, as they are known to accelerate OMB (e.g. (Pascoal et al. 2005)). The strong reduction in OMB_{micro} in agricultural and vinicultural sites, however, is likely due to a multiple stressor complex. First, in contrast to the stimulating effect of nutrients, the exceedance of nitrate levels of 10mg/L (that we found) can result in decreasing breakdown rates (cf. Woodward et al. 2012). Second, pH was found to be negatively related to OMB_{micro}. Thus, increasing pH could have reduced OMB_{micro} by reducing e.g. the fungal density (Casas and Descals 1997), as fungi are mainly responsible for OMB_{micro} (Hieber and Gessner, 2002). However, an affiliated study conducted in the same sampling region found that the negative relationship between OMB_{micro} and pH was not causal, suggesting co-variation with (an)other not measured environmental variable(s) actually affecting microbial breakdown (Fernández et al. 2015). Furthermore, it remains open whether changes in the functional composition of the communities lead to reductions in $\mathrm{OMB}_{\mathrm{micro}}$, as the response of ecosystem functions depends rather on changes in the functional than the taxonomic composition of communities (Vandewalle et al. 2010).

Regarding the invertebrate-mediated OMB ($\mathrm{OMB}_{\mathrm{macro}}$), breakdown rates were solely related to the percentage of riparian trees (out of a magnitude of environmental variables – refer to the end of this section). The positive relationship could be explained by a higher shredder density due to an increased supply of organic matter. However, no differences in $\mathrm{OMB}_{\mathrm{macro}}$ among land use types were found. The consistently high number of gammarids in all communities (App.C, Fig C.2) may have masked potential land use effects and kept OMB stable. Additionally, land use is associated with multiple stressors whose co-occurrence and interactions might have complicated finding clear differences among land use types and caused high variability in $\mathrm{OMB}_{\mathrm{macro}}$ (Aristi et al. 2012). For instance, $\mathrm{OMB}_{\mathrm{macro}}$ could have varied due to (i) different sediment loadings associated with the sandstone geology of all catchments, (ii) differences in densities or biomass of shredders (Hagen et al. 2006) which could have varied unrelated to land use across the different stream catchments or (iii) the spatial proximity of all sampling sites to the nature park may have resulted in the amelioration of land use effects, as suggested in other studies (Schäfer et al. 2012, Orlinskiy et al. 2015). Besides, functional redundancy within all invertebrate communities can additionally explain a lack of differences among land use types in $\mathrm{OMB}_{\mathrm{macro}}$ (Cadotte et al. 2011).

We determined total taxonomic richness (TTR), Simpson diversity (SD), functional diversity (FD) and species traits to investigate land use effects on $\mathrm{OMB}_{\mathrm{macro}}$ through changes in community structure. However, neither TTR, nor OMB-relevant FD (i.e. the measure of Rao's quadratic entropy (RQE) for each trait; for details refer to section 4.3.4) or their trait modalities were related to $\mathrm{OMB}_{\mathrm{macro}}$, reflecting again the dominance of gammarids. However, more heterogeneous shredder communities can occur earlier in the year, as invertebrates were sampled in autumn and by then several species have already emerged. Moreover, also homogenous habitats can explain the lack of a relationship between $\mathrm{OMB}_{\mathrm{macro}}$ and OMB-relevant trait modalities. In this context, (Frainer et al. 2014) found that traits of decomposers were linked to the most complex habitats, which again had the highest breakdown rates. By contrast, our sampling sites had similar microhabitats given dominating sandstone geology in our study area (cf. chapter 2). We found a negative, although weak, relationship between SD and OMB indicating that higher diversity resulted in lower OMB. Communities with low SD showed highest abundance of gammarids, which in turn was positively correlated with OMB (cf. App. C, Fig. C.1b), highlighting the above-mentioned compensating effect of gammarids dominance on OMB. Moreover, it is in agreement with Dangles and Malmqvist (2004) or Tolkkinen et al. (2013) who found that species dominance (including functional dominance) rather than species richness per se can be an important driver of ecosystem functioning. Additionally, it demonstrates that a dominant trait level of a single or just a few traits are needed to maintain ecosystem functioning (Gagic et al. 2015). Thus, species dominance (one mechanism on which B-EF relationships are based; Cadotte et al. 2011) can counteract the propagation of threats on organisms by land use associated stressors to ecosystem functioning. However, this does not necessarily imply that species dominance alone is sufficient to structurally and functionally stabilize ecosystems. Generally, species richness provides a buffer against environmental disturbances (i.e. insurance hypothesis; Loreau et al. 2001) and forms the basis for the recovery from these disturbances (i.e. resilience; Hillebrand et al. 2008). Beside resilience, resistance (i.e. the ability to withstand disturbances) is conceptually the second component of the relative stability of an ecosystem or community; where resilience increases with increasing species richness and resistance with increasing species dominance (Hillebrand et al. 2008). Following this conceptual model (Hillebrand et al. 2008), sampling sites of the present thesis are characterized by stream communities of low stability due to high species dominance but low species richness. This emphasizes the importance of both components in structurally and functionally stabilizing ecosystems whereas each contribution is highly complex depending on e.g. (synergistic) interspecific interactions or the identity of the dominant species (Hillebrand et al. 2008).

Finally, although our results confirm that anthropogenic land use leaves a fingerprint in the physicochemistry and habitat structure of streams, these environmental variables were just marginally related to land use categories. We gathered data on OMB of 26 catchments (in total approximately 1,446 km^2) and found that the variation in environmental variables and OMB itself was too high (within as well as among land use categories) to establish a tight relationship with land use on a regional scale. Moreover, out of a multitude of environmental variables only pH and percent tree cover were related to microbial and invertebrate-mediated breakdown, respectively. This indicates that OMB responded to stressor gradients rather than directly to land use. Thus, long-term monitoring should focus on specific stressors independent from land use categories.

5.3 EFFECTS OF LAND USE ASSOCIATED STRESSOR-GRADIENTS ON BIODIVERSITY

Changes in stream biodiversity, including taxonomic and functional diversity of invertebrate communities, were analyzed along a stressor gradient in a region of contrasting land use (chapter 4 and Appendix C). As already discussed above, land use effects on the OMB were indirectly buffered by mechanisms such as species dominance or functional redundancy. However, the stressor gradient affected the invertebrate community regarding their structure, composition of bio-ecological traits and total taxonomic richness, while both SD and FD showed no relationship with the stressor gradient.

The vulnerability of freshwater invertebrates to changes in environmental conditions varies across and within taxa (Strayer and Dudgeon 2010). As hypothesized, we found changes in the taxonomic structure that were accompanied by changes in trait modalities (e.g. taxa preferring oligotrophic conditions decreased whereas taxa preferring meso- or eutrophic conditions increased in communities along the increasing stressor gradient). This indicates environmental filtering for different traits at the opposite ends of the gradient as underlying mechanism structuring communities. However, the taxonomic and the trait structure both explained only low part of the variance. This can be due to considering only one environmental gradient (stress gradient) and to other factors structuring invertebrate communities, such as stochasticity, the spatial context or interspecific relationships (Loreau et al. 2003, Kraft et al. 2015). For example, the occurrence of a species within a community can be the result of their competitive abilities (Malmqvist 2002), colonization and extinction dynamics (i.e. neutral model; Leibold et al. 2004), random migration or immigration from neighboring populations (i.e. mass effects). Our results are in agreement with other studies reporting functional homogenization due to land-use related stressors (i.e. nutrient enrichment; Feio et al. 2015). Moreover, hydromorphological changes and urbanization within catchment areas have been suggested to modify the taxonomic structure of invertebrate communities (Feld and Hering 2007, Aschonitis et al. 2016).

Shifts in the taxonomic structure were also accompanied by changes in the TTR of invertebrate communities along the stress gradient. Particularly, TTR decreased with increasing anthropogenic stress. This result support the suggestion of environmental filtering as community structuring mechanism. Moreover, this indicates that sensitive taxa, such as *Sericostoma spec.* and *Protonemura spec.*, were excluded from sites with higher anthropogenic stress without compensation by tolerant taxa. However, none of these shifts propagated to OMB. We attributed this to the high abundance of gammarids, as already described in the former section. Their high abundance is also the reason finding no relationship between the SD of invertebrate communities and the stress gradient, as the SD is a measure of dominance being heavily weighted towards the most abundant taxa (gammarids in our study, see) and less sensitive to changes in rare species (Magurran 2004; cf. App. C Fig. C.1a and Fig. C.2).

Shifts in the trait structure were not accompanied by changes of RQEs along the gradient (i.e. the measure of FDs for each trait). This indicates no or low functional divergence within communities along the gradient (Woodward et al. 2015), although 25% of trait modalities were significantly related to anthropogenic stress (partly owed to contrasting relationships of trait modalities within a trait: cf. App. C Tab. C.6). This supports the interpretation of high functional dominance in the last section. However, potential changes in FDs may have been masked by the resolution of the trait information used in this study (Tachet database; Usseglio-Polatera et al. 2000), potentially ignoring trait differences on the species-level (Bêche and Statzner 2009). For instance, a shift in gammarid species along the gradient was found which was not reflected in the trait values (as all species were assigned to the same traits) although it indicates different habitat demands. Contrary to studies in vegetation ecology that often directly measure traits (e.g. Pérez-Harguindeguy et al. 2013), freshwater invertebrate traits are often assigned from databases resulting in the ignorance of intraspecific variability and adaptations. This could impede the detection of responses to anthropogenic stress (but see Shipley et al. 2016). Moreover, another methodical artifact could result from information loss associated with calculating a single trait diversity value out of multiple trait modalities (Dolédec and Statzner 2010), of which several individual traits responded to the stress gradient. Furthermore, the length of the stress gradient may have been too short to allow for the detection of potential effects.

Thus, for the TTR we can confirm the hypothesis, that the taxonomic diversity would be reduced when anthropogenic stress would increase – whereas for SD it has to be rejected. Although organism traits (as FD metrics) were suggested as reliable indicators for complementing current ecosystem assessment or assessing the integrity of ecosystem functions (Cadotte et al. 2011), we did not find evidence supporting our hypothesis of declining FD with increasing anthropogenic stress.

5.4 CONCLUSIONS & FUTURE CHALLENGES

Following our results, we conclude that real-world situations in freshwater ecosystems are highly complex. This complexity impedes the assessment of chemical's toxicities to, the capture of land use effects and associated stressors on and the prediction of changes in functional endpoints, respectively.

First, safety factors of 100 or 10 of the EC50 for *D. magna* or *P. subcapitata*, respectively, may not be sufficient for pesticides to protect functional endpoints on the first tier. The establishment of effect thresholds (based on concentration-effect relationships) that are protective for as many components of an ecosystem as possible is an ongoing challenge which is impeded by the high variability within and between studies. Thus, if ecosystem functions are to be included as protection goal in chemical risk assessment standardized methods (in e.g. environmental conditions) are required. Moreover, a complementary alternative could be a trait-based approach that can assess species sensitivity towards a certain stressor (e.g. Malaj et al. 2012) and be incorporated as an important tool into the ecological risk assessment (Van den Brink et al. 2016). Hence, future challenges will be to validate the recommended approaches regarding their suitability for the holistic protection of freshwater ecosystems. This includes the biggest challenge: acquiring greater knowledge on the interactions of multiple stressors in freshwater ecosystems (Van den Brink et al. 2016).

Second, we conclude that simplifying real-world stressor gradients into few land use categories was unsuitable to predict and quantify losses of ecosystem functions and services on regional or larger scales, which is often done for terrestrial ecosystems in ecosystem service assessment (Maes et al. 2012). When assessing ecosystem services is of main interest the monitoring of specific stressors may be more relevant than crude land use categories to detect effects on ecosystem functions. However, this may limit the large scale assessment of the status of OMB. Consequently, a tight relationship between land use and environmental variables or OMB has to be established to allow for extrapolation on larger scales (as desired, for instance, in the context of the WFD). Beyond the mentioned challenge to disentangle multiple stressor interactions additional investigations on real-world ecosystem dynamics at large spatial and long temporal scales are required (Brose and Hillebrand 2016). In this context, the functional resistance due to species dominance observed in the present thesis might be a short-term effect and long temporal monitoring is needed to assess the long-term resilience of stream communities to anthropogenic stress in the considered sampling area (cf. Brose and Hillebrand 2016). However, since mechanisms regulating B-EF relationships were mainly investigated in experimental studies (Balvanera et al. 2006) more field studies are needed validating mechanisms' relevance to the spatial and temporal complexity of natural ecosystems (Duffy 2009). In this context, ecologists call for the inclusion of multiple ecosystem functions, food web interactions and measuring traits continuously into field studies of B-EF relationships (Reiss et al. 2009, Gamfeldt et al. 2013).

Third, our results show that neither taxonomic nor functional diversity were suitable predictors of ecosystem functioning. Thus, when understanding anthropogenic impacts on the linkage between biodiversity and ecosystem functioning is of main interest, focusing on diversity metrics that are clearly linked to the stressor in question (Jackson et al. 2016) or that incorporates taxonomic and functional metrics (Mondy et al., 2012) might be better predictors. Furthermore, leading ecologists rather suggest a combination of different tools for future assessment of freshwater biodiversity and ecosystem services (Jackson et al. 2016). Jackson and colleagues recommend that novel tools should include remote sensing (offering global coverage data), molecular tools (based on DNA or RNA markers; e.g. metabarcoding of pooled invertebrate samples) and local-to-global citizen science (i.e. network of citizen scientists being trained in monitoring techniques to assess freshwater health; Silvertown, 2016).

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APPENDIX A

Supplementary information for
Chapter 2
Review on the effects of toxicants on freshwater ecosystem functions

The Supplementary data comprises a table with all studies describing adverse effects (Table A.1), a table with reasons for the exclusion of studies (Table A.2), a table with detailed information on the studies below the effect size of 20% (Table A.3), a table reporting detailed information on the studies in A.1 (Table A.4).

Table A.1: Studies considered in the present review describing adverse effects (=reduced performance) in three ecosystem functions with respective explanatory variables and logTUs (75 observations). For further details on abbreviations see explanations below as well as chapters *Minimum effect size* to *Calculation of toxic units* in the main text. For detailed logTU calculations we refer to Table A.3.

Reference	Study system[a]	Ecosystem type[b]	Ecosystem function[c]	Organism group[d]	Toxicant type[e]	% Reduction to control	Exposure scenario[f]	Exposure time (days)	Measured value (affected site)	Measured value (control)	Unit of measured value	logTU
Daam et al., 2010	3	1	1	2	4	26	1	28	37	50	% mass loss	-1.886490725
van Wijngaarden et al., 2004	2	2	1	1	7	40	2	8	30	50	% mass loss	-0.54
Cuppen et al., 2002	2	2	1	1	6	28	2	8	47	65	% mass loss	-1.00
Cuppen et al., 2002	2	2	1	2	6	21	2	28	65	82	% mass loss	-3.162588977
Moreirinha et al., 2011	2	2	1	2	1	23	2	14	43	56	% mass loss	2.053875381
Moreirinha et al., 2011	2	2	1	2	2	50	2	14	28	56	% mass loss	-0.312811826
Moreirinha et al., 2011	2	2	1	2	2	39	2	14	34	56	% mass loss	-0.170505019
Pradhan et al., 2011	2	2	1	2	1	43	2	21	0.0208	0.0368	day-1	2.258454483
Pradhan et al., 2011	2	2	1	2	1	36	2	21	0.0237	0.0368	day-1	4.630646834
Pradhan et al., 2011	2	2	1	2	1	40	2	21	0.0219	0.0368	day-1	1.258060922
Pradhan et al., 2011	2	2	1	2	1	41	2	21	0.0218	0.0368	day-1	3.329616839
Medeiros et al., 2010	2	2	1	2	1	32	2	16	34	50	% mass loss	0.527556851
Pascoal et al., 2010	2	2	1	2	1	25	2	21	27	36	% mass loss	1.990454682
Pestana et al., 2009	3	1	1	1	6	28	2	20	0.0093	0.013	day-1	0.32
Rasmussen et al., 2008	3	1	1	1	6	71	1	0.0625	0.05	0.17	g	-1.53
Kreutzweiser et al., 2008	2	2	1	1	6	36	1	14	25	39	% mass loss	0.15
Duarte et al., 2008a	2	2	1	2	1	22	2	40	0.0209	0.0269	day-1	1.387750815
Duarte et al., 2004	2	2	1	2	1	20	2	25	74	93	% mass loss	0.689424686
Duarte et al., 2009	2	2	1	2	1	29	2	30	55	78	% mass loss	2.640438226

Duarte et al., 2009	2	2	1	2	1	31	2	30	54	78	% mass loss	1.689424686
Duarte et al., 2009	2	2	1	2	1	33	2	30	52	78	% mass loss	2.686519696
Medeiros et al., 2008	1	1	1	1	1	44	2	7	10	18	% mass loss	-1.64
Medeiros et al., 2008	1	1	1	1	1	23	2	30	41	53	% mass loss	-1.98
Medeiros et al., 2008	3	1	2	2	1	44	2	30	0.5	0.89	mgO2/h/g	-0.927315118
Schäfer et al., 2007	1	1	1	1	7	57	1	2	0.0252	0.0588	day-1	-2.11
Carlisle and Clements, 2005	1	1	1	1	1	64	2	171	-0.002	-0.0055	day-1	-3.59
Carlisle and Clements, 2005	1	1	2	2	1	27	2	2	2.1	2.87	µgO2h-1mgDM-1	-0.85036867
Kashian et al., 2004	3	1	2	1	1	29	1	10	0.6	0.84	mgO2/L	-2.66
Wilson et al., 2004	3	2	2	3	3	20	2	35	0.0048	0.006	mgO2/day	-1.69255929
Widenfalk et al., 2004	2	2	2	2	4	22	2	8	84	108	ugCO2/(g*d)	-4.030584088
Widenfalk et al., 2004	2	2	2	2	6	32	2	4	34	50	ugCO2/(g*d)	-5,221849
Widenfalk et al., 2004	2	2	2	2	6	27	2	1	36	49	ugCO2/(g*d)	-4.191789027
Clements, 2004	3	1	2	1	1	23	1	10	0.54	0.7	mgC/LO2	-3.33
Hill et al., 1997	1	1	3	3	1	67	2	4	3.35	10.18	gO2/m2/d	-2.140403263
Hill et al., 1997	1	1	2	1	1	26	2	4	0.48	0.65	gO2/m2/d	-2.60
Villeneuve et al., 2011	3	1	3	3	7	23	2	3	3.7	4.8	ugC/h/cm2	0.131404779
Pannard et al., 2009	2	2	3	3	5	39	2	7	5.3	8.7	ugC/L/h	-3.155335851
Bhattacharyya et al., 2000	2	2	3	3	1	20	2	0.167	80	100	% mass loss	1.048451073
Bhattacharyya et al., 2000	2	2	2	3	1	20	2	0.167	80	100	% mass loss	1.979349981
Schneider et al., 1995	3	1	3	3	5	20	2	0.167	80	100	% mass loss	-0.714209972
Crossland et al., 1991	3	1	3	3	6	20	2	28	19.24	24.05	gO2/m3/h	-3.500312917

Schultheis et al., 1997	1	1	1	1	1	45	2	84	-0.016	-0.029	day-1	-1.62
Hedtke, 1984	2	2	3	3	1	28	2	28	224	309	mgC/2wk microcosm	-1.773456131
Hedtke, 1984	2	2	2	1	1	26	2	28	409	550	mgC/2wk microcosm	-2.38
Gray and Ward, 1983	1	1	1	1	1	47	2	28	17	32	% mass loss	0.00
Leland and Carter, 1985	3	1	3	3	1	20	2	8	1.25	1.57	mgC/m2/h	-2.343999022
Chaffin et al., 2005	1	1	1	1	1	42	2	102	0.0052	0.0089	day-1	-1.73
Chaffin et al., 2005	1	1	1	1	1	60	2	144	0.0019	0.0048	day-1	-1.73
Roussel et al., 2008	3	1	1	1	1	38	2	49	0.0053	0.0086	day-1	-1.58
Schäfer et al., 2012	1	1	1	1	7	20	1	2	0.00104	0.0013	day-1	-2.70
Sridhar et al., 2001	1	1	1	1	1	84	2	42	0.009	0.055	day-1	1.33
Cuffney et al., 1990	1	1	1	1	6	52	1	0.167	-0.0049	-0.0102	day-1	0.41
Cuffney et al., 1990	1	1	1	1	6	56	1	0.167	-0.0017	-0.0039	day-1	0.41
Newman et al., 1987	3	1	1	1	2	41	2	35	0.044	0.074	day-1	0.88
Wallace et al., 1986	1	1	1	1	6	28	2	385	0.0229	0.0316	day-1	2.30
Maltby et al., 1995	1	1	1	1	1	53	1	2	15	32	% mass loss	-1.01
Maltby et al., 1995	1	1	1	1	1	21	1	2	19	24	% mass loss	-0.47
Maltby et al., 1995	1	1	1	1	1	31	1	2	25	36	% mass loss	-1.16
Fernandes et al., 2009	2	2	1	2	1	21	2	35	62	78	% mass loss	0.851346989
Stout and Cooper ,1983	3	1	1	1	2	25	2	4	0.046	0.0615	day-1	0.76
Abel and Bärlocher, 1984	2	2	3	3	6	51	1	0.167	0.037	0.075	mgC/m3/hr	-1.257198422
Auber et al., 2011	3	2	1	1	7	57	2	28	0.01	0.023	day-1	-0.35
Auber et al., 2011	3	2	1	1	7	57	2	28	0.01	0.023	day-1	-0.36

Auber et al., 2011	3	2	1	1	7	39	2	28	0.014	0.023	day-1	-0.45
Auber et al., 2011	3	2	1	1	7	52	2	28	0.011	0.023	day-1	-0.63
Hoagland et al., 1993	3	2	3	3	5	38	2	7	0.03	0.048	mgO2/L/h	-0.854306041
Hoagland et al., 1993	3	2	3	3	6	75	2	7	0.012	0.048	mgO2/L/h	-4.614393726
Hoagland et al., 1993	3	2	3	3	7	50	2	7	0.024	0.048	mgO2/L/h	-0.853551173
Hoagland et al., 1993	3	2	3	3	7	52	2	7	0.023	0.048	mgO2/L/h	0.145701514
Herman et al., 1986	3	2	3	3	5	69	2	35	10.3	32.7	mgC/m2/h	-0.130030172
Bermingham et al., 1996	1	1	1	1	1	67	2	92	0.0065	0.0195	day-1	-0.14
Bermingham et al., 1996	1	1	1	1	1	57	2	92	0.0021	0.0049	day-1	-0.14
Bermingham et al., 1996	1	1	2	2	1	44	2	92	0.96	1.7	mg 02/mL/h/	-0.045167359
Bermingham et al., 1996	1	1	2	2	1	24	2	92	0.69	0.91	mg 02/mL/h/	-0.045167359
Rasmussen et al., 2012	1	1	1	2	7	20	1	2	0.001456	0.00182	day-1	-2.980053318

Abbreviations: a: 1=field study. 2=laboratory. 3=semi-field; b: 1=lotic. 2=lentic; c: 1=leaf litter breakdown. 2=community respiration; 3=primary production; d: 1=decomposer-detritivore community. 2=microbial decomposer community. 3=aquatic plants; e: 1=heavy metal. 2=others. 3=pharmaceuticals. 4=fungicide. 5=herbicide. 6=insecticide. 7=pesticide-mixture; f: 1=episodic. 2=chronic.

Table A.2: Reasons for the exclusion of certain studies from the present study (76 studies). For more details see chapters *Literature selection* and *Overview on studies and observed effects* in the main text.

Reason for exclusion	References
Acidic conditions	Dangles et al., 2004a; Dangles et al., 2004b; Kirby, 1992; Levings et al., 2005; Löhr et al., 2006; McCullough and Horwitz, 2010; Muniz, 1990; Petrin et al., 2008; Rowe et al., 2007; Schlief and Mutz, 2005; Baudoin et al, 2008
Consulted for additional information only (e.g. concentrations)	Krauss et al., 2001; Schäfer et al., 2011; Sridhar et al., 2000; Wallace et al., 1989
Effect size not extractable	Abelho and Graca, 2001; Boisson and Perrodin, 2006; Boonstra et al., 2011; Huber, 1993; Krauss et al., 2011; Lehmann and Rode, 2001; Maul et al., 2006; Niyogi et al., 2002b; Seidl et al., 1998
Hormesis	Caquet et al., 2007; Crossey and La Point, 1988; Goncalves et al., 2011; Mitchell et al., 1993; Perez et al., 2007
Effect smaller than 20%	Arts et al., 2006; Barnden and Harding, 2005; Brock et al., 1993; Cuppen et al., 2000; Cuppen et al., 1997; Maltby and Booth, 1991
Eutrophic conditions	Duarte et al., 2008b; Effler et al., 2001; Lecerf and Chauvet, 2008; Pascoal et al., 2005; Hill et al., 2010; Paaby and Goldman, 1992
No community observation	Niederlehner and Cairns, 1992; Sierra and Gomez, 2010; Starodub et al., 1987
Toxicant concentrations not provided	Forbes and Magnuson, 1980; Gómez et al., 2008; Kreutzweiser et al., 2011; Kreutzweiser et al., 2008; Niyogi et al., 2001; Wiener and Suchanek, 2008; Woodcock and Huryn, 2004
No control reported	Hill et al., 2002; Woodcock and Huryn, 2005
No function of interest measured	Dorigo and Leboulanger, 200[illegible] an et al., 2008; Forrow and Maltby, 2000; Gjessing et al., 1984; Hamala and Kollig, 1985; Sc[illegible] [illegible]d Sebetich, 2004; Schaller et al., 2010a; Schaller et al., 2008, 2010b; Schmidt et al., 2010; Slijkerman et al., 2005
Dissolved oxygen measured	Melendez et al., 1993

Neither lotic nor lentic freshwater	Evans-White and Lamberti, 2009; Krauss et al., 2005; Schmitt-Jansen and Altenburger, 2005
Referring to same raw data	Chung et al., 1993; Kreutzweiser et al., 2007; Niyogi et al., 2009; Niyogi et al., 2002c; Niyogi et al., 2002a; Schultheis and Hendricksa, 1999; Sridhar et al., 2005; Wallace et al., 1982

Table A.3: 6 Studies describing adverse effects (=reduced performance; below the effect size of 20%) with the respective explanatory variables. These effects were observed only for leaf litter breakdown in lotic ecosystems. For further details on abbreviations see explanations below as well as chapter *Minimum effect size* in the main text of chapter 2.

Reference	Study system[a]	Organism group[b]	Toxicant type[c]	% Reduction to control	Exposure scenario[d]	Exposure time (days)	Measured value (affected site)	Measured value (control)	Unit of measured value
Cuppen et al., 1997	2	1	3	10	2	28	28	31	%mass loss
Brock et al., 1993	2	1	4	4	1	28	82	85	% mass loss
Maltby and Booth, 1991	1	2	1	16	2	105	78	93	% mass loss
Cuppen et al., 2000	2	2	2	13	2	28	38	44	% mass loss
Arts et al., 2006	3	1	5	10	3	14	19	21	% decay rate
Barnden and Harding, 2005	1	1	1	5	2	63	70	74	% mass loss

Abbreviations: a: 1=field study. 2=laboratory. 3=semi-field.; b: 1=lotic.; b: 1=leaf litter breakdown.; c: 1=decomposer-detritivore community.; 2=microbial decomposer community.; c: 1=heavy metal. 2=fungicide. 3=herbicide. 4=insecticide. 5=pesticide-mixture; d: 1=episodic. 2=chronic. 3=multiple

Tab. A.4: Studies considered in the present publication (n=75 observations) with investigated measured substances and related 48h EC50 values and calculated toxic units (TU). For further details see chapters *Minimum effect size* and *Calculation of toxic units* in the main text.

Reference	Substance	EC[µg/L]	48h $EC50_{D.magna}$	$TU_{D.magna}$	$TU_{SumD.magna}$	48h $EC50_{P.subcapitata}$	$TU_{P.subcapitata}$	$TU_{Sum\ P.subcapitata}$	EC50 Reference
Daam et al., 2010	Carbendazim	100				7700$^{a\Pi}$	0.012987013		FOOTPRINT, 2011
van Wijngaarden et al., 2004	Fluazinam	2.7	220	0.012272727	0.290178049				FOOTPRINT, 2011
	Lambda-cyhalothrin	0.1	0.36	0.277777778					FOOTPRINT, 2011
	Asulam	5.4	58000	9.31034E-05					FOOTPRINT, 2011
	Metamitron	4.7	136466.6667	3.44406E-05					Schäfer et al., 2011
Cuppen et al., 2002	Chlorpyrifos	NA	NA	0.05	0.1				Cuppen et al., 2002
	Lindane	NA	NA	0.05					Cuppen et al, 2002
	Chlorpyrifos	0.05				570^{a*}	8.77193E-05	0.000687719	FOOTPRINT, 2011
	Lindane	1.5				2500$^{a\Phi}$	0.0006		FOOTPRINT, 2011
Moreirinha et al., 2011	Cadmium	3600				31.8^{a}	113.2075472		Benhra et al., 1997
	Phenanthrene	200				411	0.486618005		Schäfer et al., 2011
	Phenanthrene	200				411	0.486618005	0.67529725	Schäfer et al., 2011
	Cadmium	6				31.8^{a}	0.188679245		Benhra et al., 1997
Pradhan et al., 2011	Nano-Copperoxid	100000				551.5	181.3236627		Malaj et al., 2012
	Nanosilver	100000				2.34073994^{c}	42721.5336		Hiriart-Baer et al., 2006
	Copperion	10000				552	18.11594203		Van der Heever and Grobbelaar, 1996
	Silverion	5000				2.34073994^{c}	2136.07668		Hiriart-Baer et al., 2006
Goncalves et al., 2011	Uranium	27.8				NA		0.034031942	NA
	Zinc	20				829^{a}	0.024125452		De Schamphelaere et al., 2005
	Iron	240				NA			NA
	Mercury	0.64				78.5	0.008152866		Van der Heever and Grobbelaar, 1996
	Arsis	1.21				690^{c}	0.001753623		Richter, 1982

Medeiros et al., 2010	Zinc	1500				475[a]	3.157894737	3.369433198	Paixao et al., 2008
	Manganese	1100				5200[dΩ]	0.211538462		Fargasova et al.,1997
	Iron	7500				NA			NA
Pascoal et al., 2010	Zinc	13500				138[a]	97.82608696		Muyssen and Janssen, 2001
Pestana et al., 2009	Imidacloprid	17.6	85000	0.000207059					FOOTPRINT, 2011
	Imidacloprid	10	85000	0.000117647					FOOTPRINT, 2011
Rasmussen et al., 2008	Lambda-cyhalothrin	0.01065	0.36	0.029583333					FOOTPRINT, 2011
	Lambda-cyhalothrin	0.01065				300[a*]	0.0000355		FOOTPRINT, 2011
Kreutzweiser et al., 2008	Imidacloprid	12	85000	0.000141176					FOOTPRINT, 2011
Duarte et al., 2008a	Copper	2680				552	4.855072464	24.42028986	Van der Heever and Grobbelaar, 1996
	Zinc	2700				138[a]	19.56521739		Muyssen and Janssen, 2001
Duarte et al., 2004	Zinc	675				138[a]	4.891304348		Muyssen and Janssen, 2001
Duarte et al., 2009	Copper	241200				552	436.9565217	485.8695652	Van der Heever and Grobbelaar, 1996
	Zinc	6750				138[a]	48.91304348		Muyssen and Janssen, 2001
Medeiros et al., 2008	Iron	100	20604.20	0.004853379	0.022725706				Malaj et al., 2012
	Manganese	100	20985.91	0.004765102					Malaj et al., 2012
	Zinc	300	456407.37	0.000657308					Malaj et al., 2012
	Cadmium	100	96689.57	0.001034238					Malaj et al., 2012
	Lead	600	670434.18	0.000894942					Malaj et al., 2012
	Copper	10	950.50	0.010520738					Malaj et al., 2012

	Arsis	18	5120	25					Höss and Claus, 2011
	Iron	100	20604.20	0.004853379	0.010428416				Malaj et al., 2012
	Manganese	40	20985.91	0.001906041					Malaj et al., 2012
	Zinc	70	456407.37	0.000153372					Malaj et al., 2012
	Arsis	18				690^{c}	0.026086957		Richter, 1982
	Iron	100				NA		0.118218347	NA
	Manganese	40				$5200^{d\Omega}$	0.007692308		Fargasova et al.,1997
	Zinc	70				829^{a}	0.084439083		De Schamphelaere et al., 2005
Schäfer et al., 2007	See literature	NA	NA	0.007762471					Schäfer et al., 2007
Carlisle and Clements, 2005	Zinc	117	456407.37	0.00025635					Malaj et al., 2012
	Zinc	117				829^{a}	0.141133896		De Schamphelaere et al., 2005
Kashian et al., 2004	Zinc	1000	456407.37	0.002191025					Malaj et al., 2012
Wilson et al., 2004	Tetracyclin	4.5				2200^{a}	0.002045455		VSDB, 2011
	Oxytetracyclin	4.67				342^{a}	0.013654971	0.017252038	VSDB, 2011
	Chlortetracyclin	4.81				3100^{a}	0.001551613		VSDB, 2011
	Doxycyclin	4.86				NA			NA
	Tetracyclin	5.3				2200^{a}	0.002409091		VSDB, 2011
	Oxytetracyclin	5.5				342^{a}	0.016081871	0.020297414	VSDB, 2011
	Chlortetracyclin	5.6				3100^{a}	0.001806452		VSDB, 2011
	Doxycyclin	5.7				NA			NA
Widenfalk et al., 2004	Captan	0.11				1180^{a*}	9.32203E-05		FOOTPRINT, 2011
	Deltamethrin	0.000003				0.5^{aY}	0.000006		Caquet et al., 1992

	Isoproturon	0.32				382	0.000837696		Schäfer et al., 2011
	Pirimicarb	9				140000	6.42857E-05		Schäfer et al., 2011
Clements, 2004	Zinc	202.5	456407.37	0.000443683	0.00046747				Malaj et al., 2012
	Cadmium	2.3	96689.57	2.37875E-05					Malaj et al., 2012
Hill et al., 1997	Zinc	6	456407.37	1.31462E-05	0.002519248				Malaj et al., 2012
	Manganese	20	20985.91	0.00095302					Malaj et al., 2012
	Iron	32	20604.20	0.00155308 1					Malaj et al., 2012
	Zinc	6	456407.37	1.31462E-05	0.002519248				Malaj et al., 2012
	Manganese	20	20985.91	0.00095302					Malaj et al., 2012
	Iron	32	20604.20	0.001553081					Malaj et al., 2012
Villeneuve et al., 2011	Diuron	2.7				2	1.35	1.353333333	Schäfer et al., 2011
	Azoxystrobin	1.2				360	0.003333333		Schäfer et al., 2011
Pannard et al., 2009	Atrazine	0.1				143	0.000699301		Schäfer et al., 2011
Perez et al., 2007	Glyphosate	6000				$4400^{a\Omega}$	1.363636364		FOOTPRINT, 2011
Bhattacharyya et al., 2000	Copper	52.5				552	0.095108696	11.18023862	Van der Heever and Grobbelaar, 1996
	Zinc	52.5				829^{a}	0.063329312		De Schamphelaere et al., 2005
	Lead	107				10.35^{b}	10.33816425		Chen and Lin, 1997
	Cadmium	141				206.25	0.683636364		Van der Heever and Grobbelaar, 1996
	Copper	32				552	0.057971014	95.35642945	Van der Heever and Grobbelaar, 1996
	Zinc	199				829^{a}	0.240048251		De Schamphelaere et al., 2005
	Lead	955				10.35^{b}	92.2705314		Chen and Lin, 1997
	Cadmium	575				206.25	2.787878788		Van der Heever and Grobbelaar, 1996
Schneider et al., 1995	VelparL - Hexazinone	2.8				14.5^{a}	0.193103448		FOOTPRINT, 2011

Crossland et al., 1991	Lindane	0.79				2500[a‡]	0.000316		FOOTPRINT, 2011
Crossey and La Point, 1988	Cadmium	17.5				206.25	0.084848485	1.739431934	Van der Heever and Grobbelaar, 1996
	Copper	25.3				552	0.045833333		Van der Heever and Grobbelaar, 1996
	Nickel	16.5				143[b]	0.115384615		Chen et al., 1997
	Zinc	1238				829[a]	1.493365501		De Schamphelaere et al., 2005
Schultheis et al., 1997	Copper	23	950.50	0.024197697					Malaj et al., 2012
Hedtke, 1984	Copper	9.3				552	0.016847826		Van der Heever and Grobbelaar, 1996
	Copper	4	950.50	0.004208295					Malaj et al., 2012
Gray and Ward, 1983	Iron	9000	20604.20	0.436804107	0.996021681				Malaj et al., 2012
	Zinc	16000	456407.37	0.035056401					Malaj et al., 2012
	Manganese	11000	20985.91	0.524161173					Malaj et al., 2012
Leland and Carter, 1985	Copper	2.5				552	0.004528986		Van der Heever and Grobbelaar, 1996
Mitchell et al., 1993	Lindane	4				2500[a‡]	0.0016		FOOTPRINT, 2011
Chaffin et al., 2005	Arsis	96	5120	0.01875					Höss and Claus, 2011
Roussel et al., 2008	Copper	25	950.50	0.026301844					Malaj et al., 2012
Schäfer et al., 2012	See literature	NA	NA	NA	0.002				Schäfer et al., 2012
Sridhar et al., 2001	Arsis	3	5120	0.000585938	21.25598398				Höss and Claus, 2011
	Cadmium	2800	96689.57	0.028958657					Malaj et al., 2012
	Copper	13250	950.50	13.93997746					Malaj et al., 2012
	Iron	50	20604.20	0.002426689					Malaj et al., 2012
	Manganese	19100	20985.91	0.9101344					Malaj et al., 2012
	Nickel	2200	3265.29	0.67375415					Malaj et al., 2012
	Lead	1900	670434.18	0.002833984					Malaj et al., 2012
	Stibium	100	154439.35	0.0006475					Malaj et al., 2012

				03		
	Zinc	2600000	456407.37	5.696665192		Malaj et al., 2012
Cuffney et al., 1990	Methoxychlor	128	50	2.56		Schäfer et al., 2011
Newman et al., 1987	Sodium Hypochlorite	250	33	7.575757576		FOOTPRINT, 2011
Wallace et al., 1986	Methoxychlor	10000	50	200		Schäfer et al., 2011
Maltby et al., 1995	Aluminum	131.5	11811.43	0.011133279	0.09800686	Malaj et al., 2012
	Iron	590	20604.20	0.028634936		Malaj et al., 2012
	Chromium(VI)	4	715.86	0.005587689		Malaj et al., 2012
	Lead	42.1	670434.18	6.27951E-05		Malaj et al., 2012
	Nickel	9	3265.29	0.002756267		Malaj et al., 2012
	Copper	47.2	950.50	0.049657882		Malaj et al., 2012
	Cadmium	1	96689.57	1.03424E-05		Malaj et al., 2012
	Zinc	74.7	456407.37	0.00016367		Malaj et al., 2012
	Aluminum	112.9	11811.43	0.009558534	0.342612855	Malaj et al., 2012
	Iron	3850	20604.20	0.18685509		Malaj et al., 2012
	Chromium(VI)	3.6	715.86	0.00502892		Malaj et al., 2012
	Lead	30	670434.18	4.47471E-05		Malaj et al., 2012
	Nickel	15.5	3265.29	0.004746904		Malaj et al., 2012
	Copper	128.6	950.50	0.135296687		Malaj et al., 2012
	Cadmium	1	96689.57	1.03424E-05		Malaj et al., 2012
	Zinc	489.1	456407.37	.00107163		Malaj et al., 2012

	Aluminum	80	11811.43	0.006773098	0.068580799				Malaj et al., 2012
	Iron	470	20604.20	0.022810881					Malaj et al., 2012
	Chromium(VI)	3.1	715.86	0.004330459					Malaj et al., 2012
	Lead	30	670434.18	4.47471E-05					Malaj et al., 2012
	Nickel	11.8	3265.29	0.003613772					Malaj et al., 2012
	Copper	29.2	950.50	0.030720554					Malaj et al., 2012
	Cadmium	1	96689.57	1.03424E-05					Malaj et al., 2012
	Zinc	126.4	456407.37	0.000276946					Malaj et al., 2012
	Aluminum	80				1000000	0.00008	3.194563978	Van Hoecke et al., 2011
	Iron	470				NA			NA
	Chromium	3.1				970	0.003195876		Paixao et al., 2008
	Lead	30				10.35^b	2.898550725		Chen and Lin, 1997
	Nickel	11.8				143^b	0.082517483		Chen et al., 1997
	Copper	29.2				552	0.052898551		Van der Heever and Grobbelaar, 1996
	Cadmium	1				206.25	0.004848485		Van der Heever and Grobbelaar, 1996
	Zinc	126.4				829^a	0.152472859		De Schamphelaere et al., 2005
Fernandes et al., 2009	Zinc	980				138^a	7.101449275		Muyssen and Janssen, 2001
Stout and Cooper, 1983	4-Methylphenol	8000	1400	5.714285714					Parkhurst et al., 1979
Abel and Bärlocher, 1984	Dimethoate	5000				90400^{a*}	0.055309735		FOOTPRINT, 2011
Auber et al., 2011	Azoxystrobin	1.49	230	0.006478261	0.449802427				Schäfer et al., 2011
	Bifenthrin	0.046	0.11	0.418181818					FOOTPRINT, 2011
	Diflufenican	0.67	240	0.002791667					FOOTPRINT, 2011
	Isoproturon	12.9	580	0.0222413					FOOTPRINT, 2011

				79				
	Prochloraz	0.47	4300	0.000109302				FOOTPRINT, 2011
	Azoxystrobin	1.6	230	0.006956522	0.438375088			Schäfer et al., 2011
	Bifenthrin	0.046	0.11	0.418181818				FOOTPRINT, 2011
	Diflufenican	0.57	240	0.002375				FOOTPRINT, 2011
	Isoproturon	6.2	580	0.010689655				FOOTPRINT, 2011
	Prochloraz	0.74	4300	0.000172093				FOOTPRINT, 2011
	Bromoxynil	0.19	12500	0.0000152	0.35181109			FOOTPRINT, 2011
	Cyprodinil	74.2	220	0.337272727				FOOTPRINT, 2011
	Ioxynil	0.12	3900	3.07692E-05				FOOTPRINT, 2011
	Prosulfocarb	2.87	510	0.005627451				FOOTPRINT, 2011
	Diflufenican	0.1	240	0.000416667				FOOTPRINT, 2011
	Isoproturon	4.9	580	0.008448276				FOOTPRINT, 2011
	Bromoxynil	0.2	12500	0.000016	0.232720474			FOOTPRINT, 2011
	Cyprodinil	49.1	220	0.223181818				FOOTPRINT, 2011
	Ioxynil	0.12	3900	3.07692E-05				FOOTPRINT, 2011
	Prosulfocarb	1.86	510	0.003647059				FOOTPRINT, 2011
	Diflufenican	0.12	240	0.0005				FOOTPRINT, 2011
	Isoproturon	3.1	580	0.005344828				FOOTPRINT, 2011
Hoagland et al., 1993	Atrazine	20				143	0.13986014	Schäfer et al., 2011
	Bifenthrin	0.02				822[aП]	2.43309E-05	FOOTPRINT, 2011

	Atrazine	20				143	0.13986014	0.140103449	Schäfer et al., 2011
	Bifenthrin	0.2				$822^{a\Pi}$	0.000243309		FOOTPRINT, 2011
	Atrazine	200				143	1.398601399	1.39862573	Schäfer et al., 2011
	Bifenthrin	0.02				$822^{a\Pi}$	2.43309E-05		FOOTPRINT, 2011
Herman et al., 1986	Atrazine	106				143	0.741258741		Schäfer et al., 2011
	Iron	11720	20604.20	0.568816067					Malaj et al., 2012
	Manganese	3050	20985.91	0.145335598	0.727932999				Malaj et al., 2012
Bermingham et al., 1996	Nickel	45	3265.29	0.013781335					Malaj et al., 2012
	Iron	11720				NA			NA
	Manganese	3050				$5200^{d\Omega}$	0.586538462	0.901223776	Fargasova et al.,1997
	Nickel	45				143^{b}	0.314685315		Chen et al., 1997
Rasmussen et al., 2012	See literature					NA		0.001047	Rasmussen et al., 2012

Abbreviations: NA: not available; exposure time: a=72h, b=24h, c=92h, d=not available, alternative algae species: *=*Raphidocelis subcapitata*, Π=*Scenedesmus subspicatus*, Φ=*Scenedesmus abundans*, Ω=*Scenedesmus aquadricauda*, Y=unknown species

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APPENDIX B

Supplementary information for

Chapter 3

Organic matter breakdown in streams in a region of contrasting anthropogenic land use

Supplementary data comprises a map of the study area with sampling sites and the different land use types (Figure B.1), a figure on microbial breakdown under different nitrate levels (Figure B.2) and a figure showing the relationship between invertebrate-mediated breakdown and nitrate concentrations (Figure B.3).

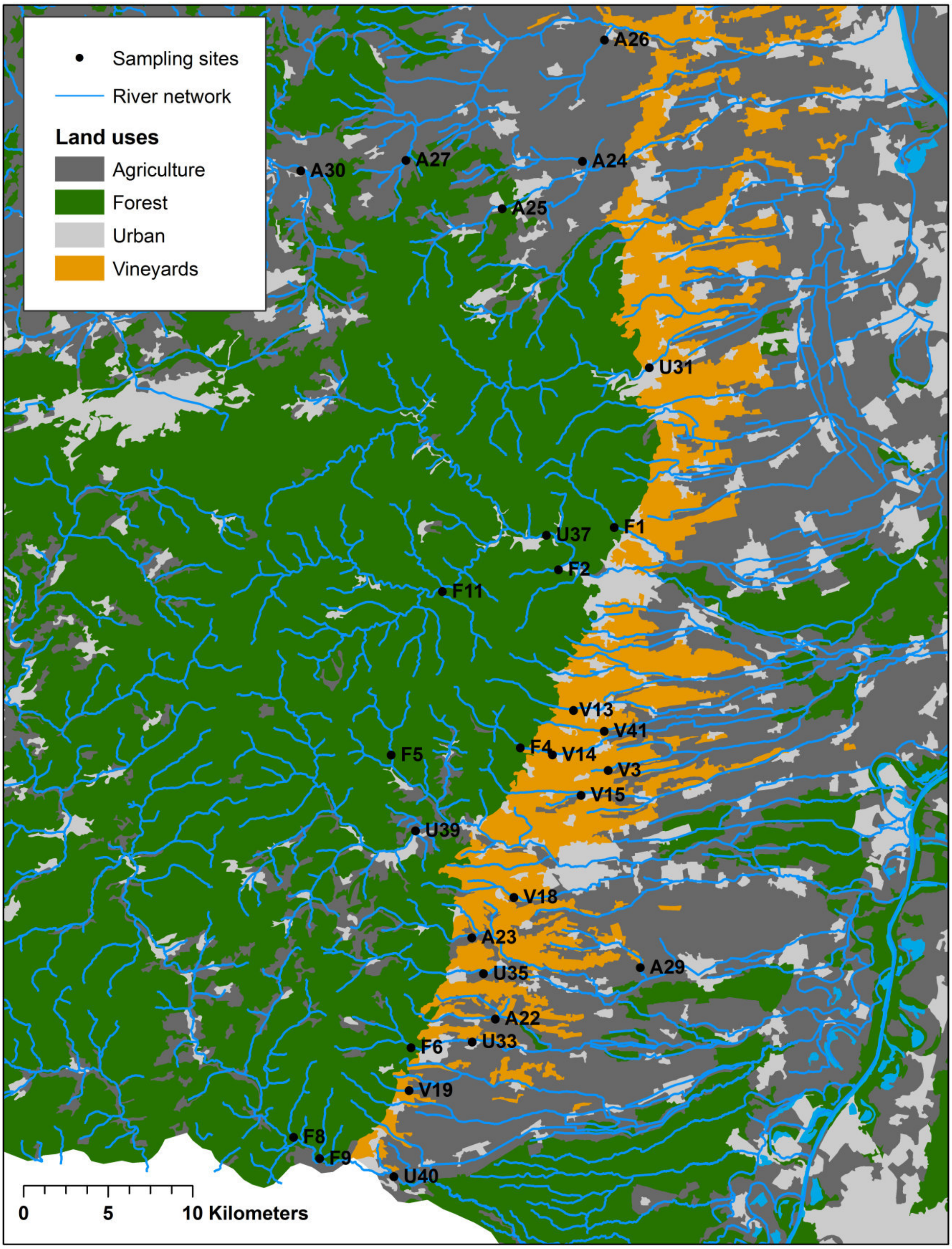

Figure B.1: Map of the study area with 29 sampling sites within the different land use types (F1-F11 = forest / reference sites; V3, V12-V19, V41=viniculture; A22-A30 = agriculture, U31-U40 = urban sites). Data on the river network for Rhineland Palatinate from (MULEWF, 2013) and Corine Land Cover from (Büttner and Kosztra, 2007).

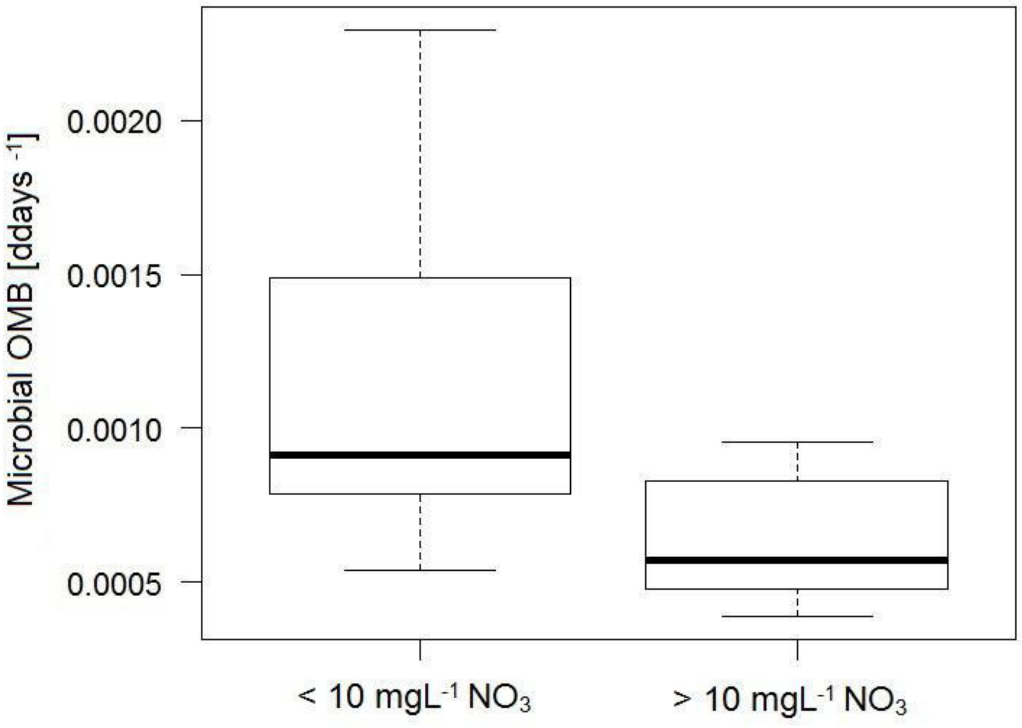

Figure B.2: Microbial breakdown per degree days (OMB_{micro}) at sampling sites with nitrate levels below 10 mgL^{-1} and above 10 mgL^{-1}. The difference is statistically significant: $p = 0.002$, Welch two sample t-test.

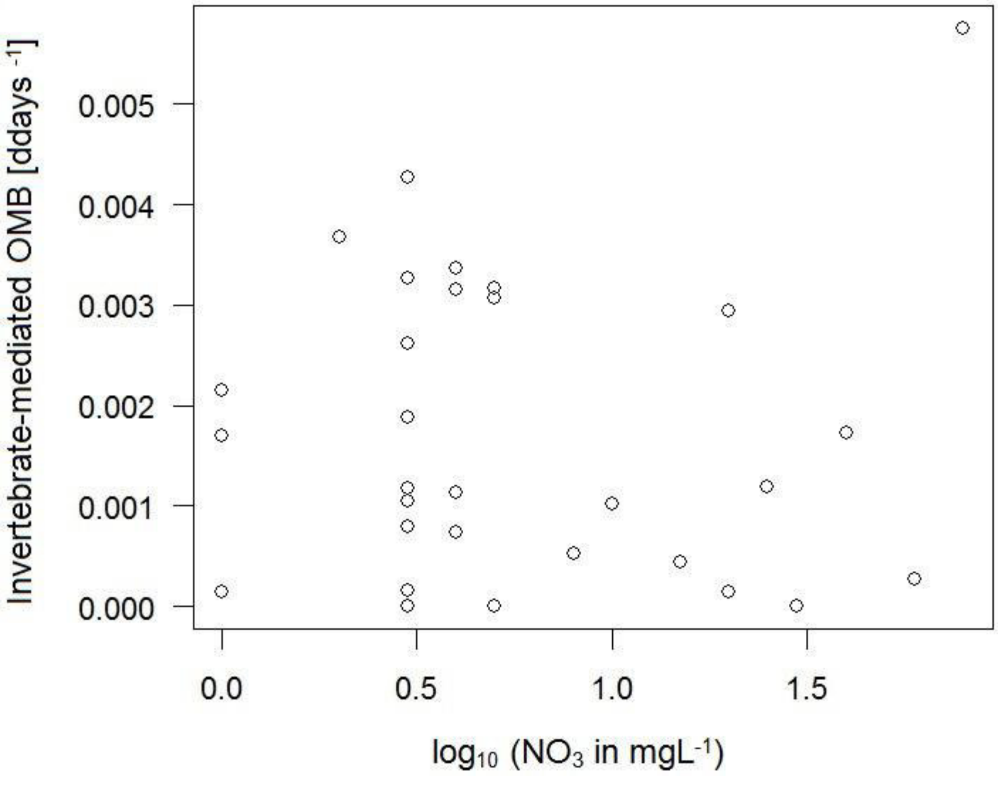

Figure B.3: Invertebrate-mediated breakdown per degree days (OMB_{macro}) against log-transformed nitrate concentrations.

APPENDIX C

Supplementary information for

Chapter 4

Taxonomic and functional diversity of stream invertebrates along an anthropogenic stress gradient

Supplementary data comprises a table of sampling site coordinates (Table C.1), of land-use related stressor gradient, OMB and the environmental variables (Table C.2), of all invertebrate taxa (Table C.3), of PCA-scores (Table C.4), of coefficient of correlations between the stressor gradient and functional diversities (Table C.5), of coefficient of correlations between the stressor gradient and 113 traits (Table C.6), of coefficient of correlations between the breakdown rate and trait modalities of OMB-relevant traits (Table C.7), a figure of relationship between the number of Gammarids and a) the Simpson diversity and b) breakdown rate *k* (Figure C.1a,b) and of the relative abundance of Gammarids at all sampling sites (Figure C.2).

Table C.1: Coordinates of 29 sampling sites.

Site No.	Land-use type	N coodinates	E coodinates
1	F	49° 22' 42.6"	08° 08' 24.2"
2	F	49° 21' 21.2"	08° 05' 46.2"
4	F	49°15'51"	08°03'27.6"
5	F	49°16'5.7"	07°57'38.6"
6	F	49°06'08"	07°58'09.8"
8	F	49°04'36.3"	07°51'37.4"
9	F	49°03'31.1"	07°52'58"
11	F	49°20'45.5"	08°00'37.7"
12	V	49°14'56.5"	08°08'20.6"
13	V	49° 16' 50.8"	08°06' 39.4"
14	V	49° 15' 25"	08° 05' 40.6"
15	V	49° 14' 7.9"	08° 06' 58.4"
18	V	49° 10' 49.7"	08° 03' 36.6"
19	V	49°04'11.4"	07°59'46.9"
22	A	49° 06' 57"	08° 03' 28.1"
23	A	49° 09' 32.7"	08° 01' 49.3"
24	A	49°34'07.2"	08°06'50"
25	A	49°32'43.4"	08°02'10.9"
26	A	49°38'19.5"	08°07'42.8"
27	A	49°34'27.4"	07°58'01"
29	A	49°08'30.2"	08°09'59.9"
30	A	49°34'02.2"	07°52'39.4"
31	U	49° 28' 34.9"	08° 10' 57.2"
33	U	49° 06' 13.4"	08° 01' 52.9"
35	U	49° 08' 26.3"	08° 02' 16.1"
37	U	49°22'26.7"	08°05'15.8"
39	U	49°12'59.1"	07°59'14.5"
40	U	49°01'52"	07°58'9.5"
41	V	49°16'09.7"	08°07'52.1"

Table C.2: The land-use related stressor gradient, OMB and the environmental variables (n = 28) characterizing the sampling sites (n = 29).

Sites	Stressor gradient	OMB [ddays^{-1}*10^3]	Stream width [m][a]	Stream depth [m][a]	Velocity [m3/s][a]	Temperature [°C][a]	pH[a]	Oxygen [mg/L][a]	Conductivity [µS/cm][a]	Nitrite [mg/L][a]	Nitrate [mg/L][a]	Phosphate [mg/L][a]	Ammonium [mg/L][a]	Megalithal (GS>40 cm)[b]	Makrolithal (GS > 20-40 cm)[b]	Mesolithal (GS > 6-20cm)[b]	Mikrolithal(GS >2-6cm)[b]	Akal (GS > 0.2-2cm)[b]	Psammal (GS > 6µm -2mm)[b]	Technolithal (artifical substrate)[b]	Algae[b]	Submerse macrophytes[b]	Emerse macrophytes[b]	Living parts of terrestrial Plants (e.g. fine roots)[b]	Xylal (e.g. woody debris)[b]	CPOM (i.e. leaf litter)[b]	Organic mud (sludge)[b]	Tree cover[c]	Riparian zone [m][d]	Shading[e]
F1	-1.12	0.16	1.67	0.15	0.47	11.25	7.56	10.13	195	0.0025	3	0.1	0	0	0	20	5	0	50	0	0	0	0	0	20	5	0	1	5.75	5
F2	-0.89	4.27	1.60	0.07	0.22	11.18	7.87	10.04	184	0.0025	3	0.1	0	0	5	25	0	0	45	0	0	0	0	0	15	10	0	1	7.5	4
F4	-0.31	0.79	2.50	0.10	0.26	11.25	7.93	9.83	229	0.005	3	0.075	0	5	10	10	5	5	50	0	0	0	0	15	0	0	0	1	10	4
F5	-0.97	0.14	1.80	0.14	0.32	11.25	7.16	10.99	59	0.0025	1	0.05	0	0	0	0	10	0	50	0	0	0	0	10	25	5	0	1	0.5	4
F6	-0.51	3.67	1.40	0.06	0.26	12.03	7.53	10.99	134	0.0025	2	0.7	0	5	0	0	5	5	50	0	0	0	5	10	10	10	0	1	5	4
F8	-1.51	2.16	2.10	0.18	0.54	12.03	7.45	11.01	127	0.0025	1	0.1	0	0	0	0	15	5	30	0	0	0	0	0	25	25	0	2	7.5	5
F9	-0.92	1.17	1.80	0.25	0.24	12.03	7.09	10.99	130	0.0025	3	0.15	0	0	0	5	0	0	10	0	0	0	60	5	20	0	0	1	4.25	3
F11	-1.62	1.05	3.80	0.40	0.77	11.25	7.01	10.92	88	0.005	3	0.15	0	5	0	35	15	0	10	5	0	10	0	0	10	10	0	2	5.25	5
V12	2.26	0.28	1.00	0.19	0.01	12.61	7.94	5.3	1290	0.1	60	0.35	0	5	0	10	0	0	10	5	0	0	0	20	0	0	50	1	1	5
V13	0.18	3.37	1.50	0.12	0.21	12.50	7.7	9.93	222	0.07	4	0.3	0	5	0	25	0	0	35	15	0	0	0	15	5	0	0	2	4	4
V14	0.58	3.15	1.70	0.11	0.10	12.66	7.72	9.25	324	0.04	4	0.2	0	10	5	20	5	5	35	5	0	0	0	10	0	0	5	1	2	5
V15	1.19	0.53	1.10	0.25	0.05	13.64	8.14	8.08	623	0.04	8	0.6	0	0	5	0	10	0	50	0	0	0	0	15	0	10	10	1	1.5	4
V18	0.94	3.07	0.80	0.12	0.12	12.68	8.03	8.19	666	0.08	5	0.4	0	5	55	5	0	0	10	5	0	0	0	15	0	5	0	2	1.5	5
V19	-0.10	1.69	1.40	0.11	0.22	12.68	7.84	10.47	284	0.015	1	0.4	0	0	0	5	0	0	55	5	0	0	0	10	0	20	5	2	1.75	5
V41	1.38	0.00	1.20	0.22	0.01	12.66	8.15	8.84	826	0.02	30	0.3	0	10	0	30	30	10	0	0	0	0	0	20	0	0	0	1	1.25	5
A22	0.55	0.43	1.50	0.17	0.08	12.62	7.97	9.6	770	0.08	15	0.2	0.02	0	0	0	0	0	20	0	0	0	0	0	20	10	50	1	3	4
A23	0.61	1.02	1.70	0.12	0.23	12.31	8.26	7.3	832	0.01	10	0.2	0	0	5	5	60	0	5	0	0	0	0	10	0	15	0	1	1.5	2
A24	1.13	1.73	1.40	0.12	0.10	13.08	7.98	8.5	1083	0.1	40	0.3	0	0	0	10	0	0	20	0	5	0	40	15	0	0	10	1	2.5	5
A25	-0.08	1.87	2.30	0.23	0.25	12.04	7.88	9.43	310	0.005	3	0.2	0	0	0	0	0	0	65	0	0	0	0	10	0	15	10	2	1.35	5
A26	0.11	5.75	1.80	0.25	0.16	12.04	8.25	10.12	947	0.02	80	0.1	0	0	0	30	25	0	15	0	0	0	0	5	10	15	0	2	3	5
A27	0.52	1.18	2.40	0.20	0.11	13.08	8.02	9.58	557	0.014	25	0.15	0	0	0	0	20	0	35	0	0	0	0	10	20	5	10	1	1.5	5

A29	1.26	2.94	1.10	0.25	0.25	12.31	8.09	8.46	1073	0.08	20	0.8	0.07	0	0	0	0	0	0	0	0	0	30	30	5	5	30	1	2.5	3
A30	0.60	0.14	2.80	0.89	0.07	13.08	7.74	9.3	530	0.1	20	0.025	0	0	10	25	5	0	30	0	0	0	0	20	0	5	5	1	2.5	5
U31	0.22	1.14	3.50	0.21	0.34	13.84	7.54	8.96	1070	0.1	4	0.1	0.2	0	0	0	0	0	70	5	0	0	0	10	0	5	10	2	2.5	4
U33	-0.36	0.00	2.40	0.28	0.35	13.03	7.51	10.61	356	0.07	5	0.15	0	5	0	60	5	5	0	0	0	0	0	5	0	20	0	2	2	4
U35	0.25	3.17	2.70	0.12	0.16	12.41	7.9	8.98	110	0.03	5	0.3	0	0	5	5	0	0	50	0	0	0	0	10	15	5	10	2	1	5
U37	-1.02	0.00	8.00	0.75	0.52	11.25	7.41	9.91	195	0.3	3	0.2	0	0	5	5	10	0	35	0	0	30	0	10	0	0	5	1	3.5	2
U39	-0.77	3.26	4.00	0.40	0.87	11.25	7.6	8.72	379	0.06	3	1	0.1	5	35	30	0	0	10	0	0	10	0	0	10	0	0	2	3	5
U40	-1.62	2.61	11.20	0.30	0.82	12.17	7.51	10.83	150	0.015	3	0.4	0	0	10	60	5	0	5	0	5	5	0	0	10	0	0	2	3	2

Letters of Sites: F = Forest, V = Vineyards, A = Agriculture, U = Urban;
OMB = Organic Matter Breakdown (invertebrate-mediated);
GS = Grain size;
CPOM = Coarse particular organic matter;
[a] = mean of three measurements;
[b] = Proportional Percentage of respective stream substrate or microhabitat in the 50-m reach of the respective sampling site, as detailed in the AQEM/STAR protocol (AQEM, 2002);
[c] = Percentage Tree cover within the riparian zone: 1 = 0 – 80% of left and right bank maximum, 2 = > 80% of left and right bank maximum;
[d] = width of riparian zone (mean of left and right bank);
[e] = Percentage of shading: 1 = < 5%, 2 = maximum of 25%, 3 = maximum of 50%, 4 = maximum of 75%. 5 = > 75%.

Tab. C.3: All invertebrate taxa found (n = 66) within the sampling period September/October 2012

Phylum	Class	Order	Family	Genus	Species
Mollusca	Bivalvia	Veneroida	Sphaeriidae	*Pisidium*	*spec.*
	Gastropoda	Architaenioglossa	Bithyniidae	*Bithynia*	*Bithynia tentaculata*
	Gastropoda	Pulmonata	Lymnaeidae	*Omphiscola*	*Omphiscola glabra*
	Gastropoda	Pulmonata	Lymnaeidae	*Radix*	*Radix ovata*
	Gastropoda	Pulmonata	Lymnaeidae	*Stagnicola*	*Stagnicola glaber*
	Gastropoda	Pulmonata	Physidae	*Physa*	*Physa foutinalis*
	Gastropoda	Pulmonata	Planorbidae	*Bathyomphalus*	*Bathyomphalus contortus*

	Gastropoda	Pulmonata	Planorbidae	*Gyraulus*	*Gyraulus albus*
	Gastropoda	Pulmonata	Planorbidae	*Hippeutis*	*Hippeutis complanatus*
	Gastropoda	Pulmonata	Planorbidae	*Planorbis*	*Planorbis planorbis*
Annelida	Clitellata	Pharyngobdelliformes	Erpobdellidae	*Erpobdella*	*Erpobdella octoculata*
	Clitellata	Rhynchobdellida	Glossiphoniidae	*Glossiphonia*	*Glossiphonia nebulosa*
	Clitellata	Rhynchobdellida	Glossiphoniidae	*Helobdella*	*Helobdella stagnalis*
	Clitellata	Rhynchobdelliformes	Glossiphoniidae	*Theromyzon*	*Theromyzon tessulatum*
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*Gammarus pulex*
	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*Gammarus roeselli*
	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*Gammarus fossarum*
	Malacostraca	Isopoda	Asellidae	*Asellus*	*Asellus aquaticus*
	Insecta	Ephemeroptera	Baetidea	*Baetis*	*spec.*
	Insecta	Ephemeroptera	Ephemeridae	*Ephemera*	*Ephemera danica*
	Insecta	Ephemeroptera	Heptageniidae	*Ecdyonurus*	*spec.*
	Insecta	Odonata	Calopterygidae	*Calopteryx*	*Calopteryx virgo*
	Insecta	Odonata	Cordulegastridae	*Cordulegaster*	*Cordulegaster bidentatus*
	Insecta	Odonata	Cordulegastridae	*Cordulegaster*	*Cordulegaster boltonii*
	Insecta	Plecoptera	Leuctridae	*NA*	*NA*
	Insecta	Plecoptera	Nemouridae	*Protonemura*	*spec.*
	Insecta	Plecoptera	Perlodidae	*Arcynopteryx*	*Arcynopteryx compacta*
	Insecta	Hemiptera	Nepidae	*Nepa*	*Nepa cincerea*
	Insecta	Hemiptera	Notonectidae	*Notonecta*	*Notonecta maculata*
	Insecta	Megaloptera	Sialidae	*Sialis*	*Sialis nigripes*
	Insecta	Neuroptera	Osmylidae	*Osmylus*	*spec.*
	Insecta	Coleoptera	Dytiscidae	*Ilybius*	*spec.*
	Insecta	Coleoptera	Dytiscidae	*Platambus*	*Platambus maculatus*
	Insecta	Coleoptera	Elmidae	*Stenelmis*	*spec.*
	Insecta	Coleoptera	Gyrinidae	*NA*	*NA*
	Insecta	Coleoptera	Hydrophilidae	*Laccobius*	*spec.*

Insecta	Coleoptera	Scirtidae	*Scirtes*	*spec.*
Insecta	Trichoptera	Hydropsychidae	*Diplectrona*	*Diplectrona felix*
Insecta	Trichoptera	Hydropsychidae	*Hydropsyche*	*Hydropsyche modesta*
Insecta	Trichoptera	Limnephilidae	*Limnephilus*	*Limnephilus extricatus*
Insecta	Trichoptera	Limnephiloidea	*Silo*	*Silo pallipes*
Insecta	Trichoptera	Odontoceridae	*Odontocerum*	*Odontocerum albicorne*
Insecta	Trichoptera	Philopotamidae	*Philopotamus*	*Philopotamus montanus*
Insecta	Trichoptera	Philopotamidae	*Philopotamus*	*Philopotamus ludificatus*
Insecta	Trichoptera	Philopotamidae	*Philopotamus*	*Philopotamus variegatus*
Insecta	Trichoptera	Polycentropodidae	*Polycentropus*	*Polycentropus irroratus*
Insecta	Trichoptera	Psychomyiidae	*Psychomyia*	*Psychomyia pusilla*
Insecta	Trichoptera	Rhyacophilidae	*NA*	*NA*
Insecta	Trichoptera	Rhyacophilidae	*Rhyacophila*	*Rhyacophila aurata*
Insecta	Trichoptera	Rhyacophilidae	*Rhyacophila*	*Rhyacophila dorsalis/vulgaris etc*
Insecta	Trichoptera	Rhyacophilidae	*Rhyacophila*	*Rhyacophila fasciata*
Insecta	Trichoptera	Rhyacophilidae	*Rhyacophila*	*Rhyacophila polonica*
Insecta	Trichoptera	Rhyacophilidae	*Rhyacophila*	*Rhyacophila pubescens*
Insecta	Trichoptera	Rhyacophilidae	*Rhyacophila*	*Rhyacophila tristis*
Insecta	Trichoptera	Sericostomatidae	*Sericostoma*	*Sericostoma personatum / falvicorne*
Insecta	Diptera	Chironomidae	*NA*	*NA*
Insecta	Diptera	Limoniidae	*NA*	*NA*
Insecta	Diptera	Limoniidae	*Antocha*	*spec.*
Insecta	Diptera	Limoniidae	*Eloeophila*	*spec.*
Insecta	Diptera	Muscidae	*Limnophora*	*Limnophora aequifrons*
Insecta	Diptera	Pediciidae	*Dicranota*	*spec.*
Insecta	Diptera	Ptychopteridae	*Ptychoptera*	*spec.*
Insecta	Diptera	Ptychopteridae	*Ptychoptera*	*Ptychoptera rufocinctus*
Insecta	Diptera	Simuliidae	*NA*	*NA*
Insecta	Diptera	Tabanidae	*NA*	*NA*
Insecta	Diptera	Tipulidae	*Tipula*	*spec.*

Tab. C.4: PCA-Scores of 27 selected environmental variables for the first four axis of the Principal Component Analysis.

Environmental variables	Stressor gradient (PC 1)	PC 2	PC 3	PC 4
Stream width	-0.22	0.38	-0.08	0.04
Stream depth	-0.06	0.32	-0.12	0.06
Temperature	0.28	0.01	0.02	-0.14
pH	0.31	-0.06	0.01	0.09
Oxygen	-0.35	-0.11	0.02	0.04
Ammonium	0.02	0.13	-0.01	-0.36
Shading	0.10	-0.22	0.27	-0.14
Megalithal	0.09	0.05	0.48	0.19
Makrolithal	0.02	0.19	0.22	-0.11
Mesolithal	-0.12	0.23	0.27	0.25
Mikrolithal	0.03	-0.07	0.00	0.40
Akal	0.02	-0.12	0.32	0.34
Psammal	-0.07	-0.23	-0.05	-0.37
Technolithal	0.07	0.05	0.30	-0.28
Algea	-0.03	0.22	-0.13	0.11
Submerse macrophytes	-0.16	0.35	-0.07	0.02
Emerse macrophytes	0.03	0.02	-0.30	0.07
Living parts of terrestrial plants	0.32	0.05	-0.03	0.02
Xylal	-0.22	-0.23	-0.21	0.00
CPOM	-0.09	-0.26	0.07	-0.03
Organic mud	0.24	0.03	-0.23	-0.15
Tree cover	-0.13	0.09	0.32	-0.29
Velocity[a]	-0.36	0.17	0.03	-0.09
Nitrite[a]	0.24	0.36	0.02	-0.15
Nitrate[a]	0.31	0.09	-0.12	0.21
Phophate[a]	0.12	0.17	0.10	-0.08
Riparian zone[a]	-0.22	-0.01	0.02	0.07

[a] = after log- or double squareroot transformation because of strong skewness

Tab. C.5: Coefficient of correlations between the stressor gradient and 21 functional diversities (RQEs) of invertebrate communities
(n = 29). Each coefficient is given with its adjusted p-value, according to correction by Benjamini and Hochberg (1995).

RQEs	r	p
Body size	-0.25	0.74
Life cycle duration	-0.14	0.74
Cycles per year	-0.31	0.74
Aquatic stage	-0.05	0.87
Reproduction	-0.17	0.74
Dispersal	-0.16	0.74
Resistance forms	-0.05	0.87

Respiration	-0.14	0.74
Locomotion	-0.24	0.74
Food	-0.10	0.84
Feeding habits	0.00	0.98
Transversal distribution	-0.09	0.85
Longitudinal distribution	-0.29	0.74
Altitude	-0.21	0.74
Substrate	-0.14	0.74
Velocity	-0.10	0.84
Trophic status	-0.15	0.74
Salinity	-0.33	0.74
Temperature	-0.21	0.74
Saprobity	-0.02	0.94
pH	-0.07	0.87
Mean RQE	-0.20	0.74

Tab. C.6: Coefficient of correlations between the stressor gradient and 113 traits (of 21 grouping features) of invertebrate communities (n = 29). Each coefficient is given with its adjusted p-value, according to correction by Benjamini and Hochberg (1995).

Trait modalities	r	p
Body size modalities		
< 0.25 cm	NA	NA
> 0.25-0.5 cm	-0.25	0.32
> 0.5-1 cm	-0.48	0.03*
> 1-2 cm	-0.19	0.47
> 2-4 cm	0.73	0.00**
> 4-8 cm	-0.18	0.49
> 8 cm	NA	NA
Life cycle duration modalities		
< 1 year	-0.35	0.15
> 1 year	0.35	0.15
Cyles per year modalities		
No. of cycle: < 1	-0.40	0.08[A]
No. of cycle: 1	0.19	0.47
No. of cycle: > 1	0.14	0.60
Aquatic stage modalities		
Egg	0.15	0.60
Larva	0.10	0.72
Nymph	-0.54	0.01*
Adult	0.60	0.00*

Reproduction modalities		
Ovoviviparity	0.45	0.05^{A}
Isolated eggs free	-0.35	0.15
Isolated eggs cemented	-0.31	0.21
Clutches cemented or fixed	0.01	0.99
Clutches free	0.04	0.89
Clutches in vegetation	0.27	0.29
Clutches terrestrial	-0.41	0.08
Asexual reproduction	NA	NA
Dispersal modalities		
Aquatic passive	0.39	0.10
Aquatic active	0.60	0.00*
Aerial passive	-0.43	0.06^{A}
Aerial active	-0.44	0.06^{A}
Resistance forms		
Eggs statoblasts	-0.28	0.26
Cocoons	-0.06	0.85
Housings against desiccation	0.21	0.43
Diapause or dormancy	-0.03	0.91
None	0.15	0.60
Respiration modalities		
Tegument	0.20	0.46
Gill	-0.12	0.69
Plastron	-0.08	0.79
Spiracle	-0.03	0.89
Hydrostatic vesicle	NA	NA
Locomotion modalities		
Flier	0.14	0.60
Surface swimmer	0.42	0.07^{A}
Full water swimmer	0.63	0.00*
Crawler	-0.05	0.87
Burrower	-0.21	0.43
Interstitial	-0.11	0.69
Temporarily attached	-0.39	0.10
Permanently attached	-0.32	0.19
Food modalities		
Microorganisms	-0.06	0.85
Detritus (< 1mm)	-0.51	0.02*
Dead plant (> 1mm)	0.05	0.87
Living microphytes	-0.31	0.20
Living macrophytes	0.00	0.99

Dead animal (> 1mm)	0.67	0.00*
Living microinvertebrates	0.30	0.23
Living macroinvertebrates	-0.07	0.80
Vertebrates	0.10	0.71
Feeding habit modalities		
Absorber	NA	NA
Deposit feeder	0.05	0.87
Shredder	0.00	1.00
Scraper	-0.11	0.70
Filter-feeder	-0.54	0.01*
Piercer	0.32	0.20
Predator	0.04	0.89
Parasite	0.19	0.47
Transversal distribution modalities		
River channel	-0.59	0.01*
Banks connected side-arms	-0.15	0.60
Ponds , pools, disconnected side-arms	0.49	0.03*
Marshes, peat bogs	0.35	0.15
Temporary waters	0.11	0.69
Lakes	0.56	0.01*
Groundwaters	NA	NA
Longitudinal distribution modalities		
Crenon	-0.31	0.20
Epirithron	-0.64	0.00*
Metarithron	-0.39	0.10
Hyporithron	-0.29	0.24
Epipotamon	0.45	0.05^{A}
Metapotamon	0.62	0.00*
Estuary	0.27	0.28
Outside river system	0.35	0.15
Altitude modalities		
Lowlands	0.56	0.01*
Piedmont level	-0.14	0.60
Alpine level	-0.61	0.00*
Substrate modalities		
Flags/boulders/cobbles/pebbles	-0.11	0.69
Gravel	0.05	0.87
Sand	-0.27	0.29
Silt	-0.20	0.45
Macrophytes	0.22	0.40
Microphytes	0.35	0.15
Twigs/roots	-0.24	0.37

Organic detritus/litter	-0.16	0.56
Mud	0.18	0.50
Velocity modalities		
Null	0.54	0.01*
Slow (< 25 cm/s)	-0.19	0.47
Medium (25-50 cm</s)	-0.22	0.43
Fast (> 50 cm/s)	-0.52	0.02*
Trophic status modalities		
Oligotrophic	-0.60	0.00*
Mesotrophic	0.52	0.02*
Eutrophic	0.45	0.05^{A}
Salinity modalities		
Freshwater	-0.66	0.00*
Brackish water	0.65	0.00*
Temperature modalities		
Cold <15C	-0.54	0.01*
Warm >15C	-0.31	0.20
Eurythermic	0.59	0.01*
Saprobity modalities		
Xenosaprobic	-0.72	0.00**
Oligosaprobic	-0.71	0.00**
b-mesosaprobic	0.48	0.03*
a-mesosaprobic	0.67	0.00*
Polysaprobic	0.46	0.04*
pH modalities		
pH <4	0.17	0.52
pH >4-4.5	0.10	0.72
pH >4.5-5	-0.24	0.37
pH >5-5.5	-0.49	0.03*
pH >5.5-6	-0.15	0.60
pH >6	0.36	0.14

Abbreviations: NA: not available, * $p < 0.05$, ** $p < 0.001$, ^{A} $0.05 \leq p < 0.1$ (trend)

Tab. C.7: Coefficient of correlations between the breakdown rate and trait modalities of OMB-relevant traits. Each coefficient is given with its adjusted p-value, according to correction by Benjamini and Hochberg (1995).

OMB-relevant grouping features with their respective traits	r	p
Food RQE	-0.19	0.33
Food modalities		
Microorganisms	0.04	0.99
Detritus (< 1mm)	-0.16	0.99
Dead plant(> 1mm)	0.06	0.99
Living microphytes	-0.14	0.99
Living macrophytes	0.07	0.99
Dead animal(> 1mm)	-0.01	0.99
Living microinvertebrates	-0.40	0.49
Living macroinvertebrates	0,24	0.99
Vertebrates	0.15	0.99
Fedding habit RQE	-0.20	0.33
Feeding habit modalities		
Absorber	NA	NA
Deposit feeder	-0.10	0.99
Shredder	-0.08	0.99
Scraper	0.06	0.99
Filter-feeder	-0.17	0.99
Piercer	0.20	0.99
Predator	0.00	0.99
Parasite	-0.01	0.99

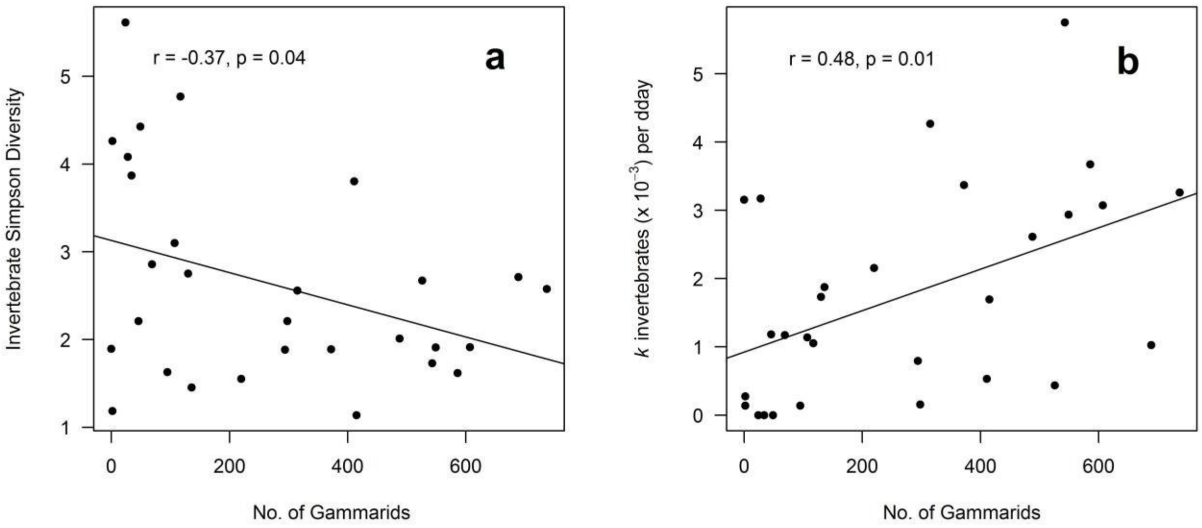

Fig. C.1: Relationship between the number of Gammarids (sum of all three *Gammarus* species) and **a)** the Simpson diversity and b) breakdown rate *k*. Regression lines are added to visualize the a) negative and **b)** positive correlation.

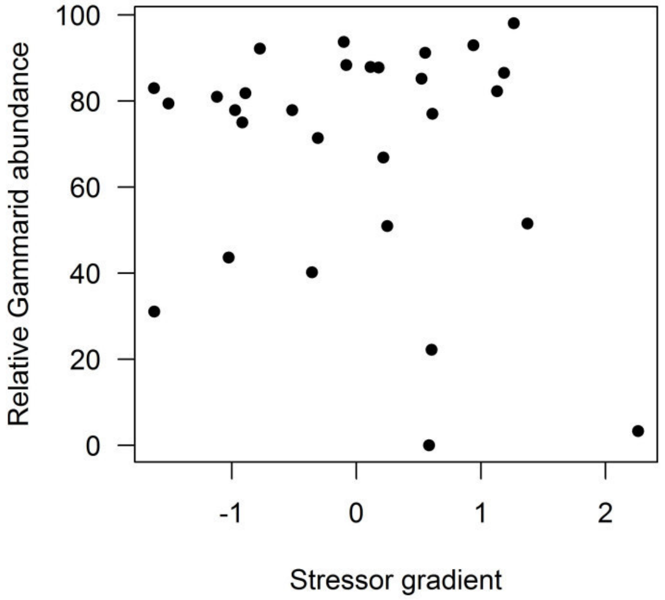

Fig. B.2: Relative abundance of Gammarids (all three *Gammarus* species) at 29 sampling sites of the stressor gradient (mean abundance 70%).

Reference:

Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57:289–300

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Publications (peer - reviewed)

Voß, K., Schäfer, R.B. 2016. Taxonomic and functional diversity of stream invertebrates along an anthropogenic stress gradient. In Review in Ecological Indicators.

Voß, K., Fernández, D., Schäfer, R.B. 2015. Organic matter breakdown in streams in a region of contrasting anthropogenic land use. Science of the total Environment (527–528): 179–184.

Fernández, D., **Voß, K.**, Bundschuh, M., Zubrod, J., Schäfer, R.B. 2015. Effects of fungicides on decomposer communities and litter decomposition in vineyard streams. Science of the Total Environment (533): 40-48.

Peters, K., Bundschuh, M., Schäfer, R.B. 2013 Review on the effects of toxicants on freshwater ecosystem functions. Environmental Pollution (180): 324-329

(non peer-reviewed)

Voß, K. Effekte anthropogener Landnutzung auf eine wichtige Ökosystemfunktion in Fließgewässern der Südpfalz. WasserWirtschaft, Sonderausgabe 12/15 zum Treffen „Wasserrahmrichtlinie 2015 - Was wurde erreicht, was bleibt zu tun?“.

Conference Presentations

Voß, K. Effekte Anthropogener Landnutzung auf eine wichtige Ökosystemfunktion in Fließgewässern der Südpfalz. Treffen „Wasserrahmrichtlinie 2015 - Was wurde erreicht, was bleibt zu tun?“.

Voß, K., Fernández, D., Schadt, S., Schäfer, R.B., (2015). Do changes in the biodiversity of freshwater communities propagate to ecosystem functioning? SEFS 09 - Symposium for European Freshwater Science, Geneva, Switzerland

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Peters, K., Fernández, D., Schadt, S., Kolb, N., Heuring, A., Schäfer, R.B. (2013). Do effects of land use on the diversity and structure of invertebrate communities propagate to a fundamental ecosystem funtion? SIL (International Society of Limnology) XXXII Congress 2013, Budapest, Hungary.

Peters, K., Bundschuh, M., Schäfer, R.B. (2013). Review on the effects of toxicants on freshwater ecosystem functions. Third Young Environmental Scientists Meeting 2013, Krakow, Poland

Peters, K., Bundschuh, M., Schäfer, R.B., (2012). Auswirkungen von Schadstoffen auf aquatische Ökosystemfunktionen. SETAC-GLB-Leipzig 2012

Author's Contributions

Paper I

Title: Review on the effects of toxicants on freshwater ecosystem functions
Authors: Katharina Peters, Mirco Bundschuh and Ralf B. Schäfer
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Contribution: Peters (70 %) Designed research, Analysed data, Discussed results, Wrote manuscript
Bundschuh (15 %) Designed research, Discussed results, Edited manuscript
Schäfer (15 %) Designed research, Discussed results, Edited manuscript

Paper II

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Authors: Katharina Voß, Diego Fernández and Ralf B. Schäfer
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Contribution: Voß (75 %) Designed research, Analysed data, Discussed results, Wrote manuscript
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Paper III

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Authors: Katarina Voß and Ralf B. Schäfer
Status: In Review in Ecological Indicators
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