Chemical stressors influence aquatic ecosystem processes

Fungicide effects on decomposer communities and primary consumers

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Cover: Detritivores feeding on leaf material
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Chemical stressors influence aquatic ecosystem processes – Fungicide effects on decomposer communities and primary consumers.

Abstract

Leaf litter decomposition is a fundamental ecosystem process for the energy provisioning in streams, mainly mediated by microbial leaf decomposers and leaf-shredding, detritivorous macroinvertebrates. Both decomposers and detritivores are under chemical stress from pesticides entering surface waters. Amongst these, fungicides may pose a particular risk, as they can negatively affect aquatic microbial decomposers but also detritivores *via* both waterborne exposure and by influencing the quality of their food. The overall objective of my thesis was to broaden the knowledge of fungicide effects on organisms and processes mediating leaf litter decomposition in streams as well as the interactions between decomposers and detritivores.

Fungicides affected microbial decomposers by altering fungal biomass and community composition, and by changing the microbial fatty acid profile. These structural effects subsequently resulted in effects on microbial leaf litter decomposition. However, the strength of functional responses was dependent on the exposure history of microorganisms to chemical stressors, with previously exposed organisms showing less negative or even positive responses to fungicide exposure. Such a functional adaptation of microbial decomposers to chemical stress was congruently observed on a larger biogeographical scale within Europe, despite distinct structural responses at the individual study sites. Moreover, fungicides caused indirect effects on detritivores by reducing the palatability of leaf material and affecting the food choice of detritivores. Structural alterations on the microbial level led to a reduced food quality of leaf litter. Feeding on leaf litter of lower quality ultimately affected detritivores' food processing (consumption and excretion) and resulted in lower lipid content and growth. Similar effects, although more pronounced, were observed for detritivores directly exposed through water. Nevertheless, neither effect pathway should be ignored given their additive action.

Risks for fungicide effects at the base of the aquatic food web under field conditions can be expected, since effects on decomposers and detritivores were observed at field-relevant fungicide concentrations during this thesis. These findings in combination with the predicted higher fungicide use in the future due to agricultural intensification are reasons for concern, given the central roles of decomposers and detritivores in aquatic ecosystem functioning.

Keywords: Bacteria, Detritivores, Detritus, Direct effects, Fungi, Indirect effects, Leaf litter decomposition, Shredder, Trophic interaction

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Kemiska faktorer inverkar negativt på processer i akvatiska ekosystem – Effekter av fungicider på nedbrytarsamhällen och primärkonsumenter

Sammanfattning

Lövnedbrytning är en grundläggande ekosystemprocess som påverkar näringstillgången i vattendrag. Processen utförs främst av makroevertebrater som fragmenterar löven (detritivorer) och mikrober som bryter ner löven. Dessa båda organismgrupper kan emellertid påverkas av kemiska föroreningar som hamnat i vattnet. Bland dessa kan fungicider utgöra en särskild risk. De påverkar främst mikrobiella nedbrytare, men kan även ha negativ inverkan på detritivorer genom direkt exponering via vattnet och genom att påverka kvalitén på deras föda. Det övergripande målet med min avhandling var att öka kunskapen om fungiciders effekter på de organismer och processer som medverkar till lövnedbrytning i vattendrag, samt om interaktioner mellan detritivorer och nedbrytare.

Fungicider påverkade mikrobiella nedbrytare genom att förändra svampbiomassan, svampsamhällets artsammansättning och den mikrobiella fettsyresammansättningen. Dessa strukturella effekter påverkade i sin tur den mikrobiella lövnedbrytningen. Effekterna var emellertid beroende av mikroorganismernas tidigare exponering av kemikalierna, där organismer som tidigare hade utsatts visade mindre negativa, eller till och med positiva, effekter vid exponering för fungicider. En sådan anpassning till kemisk stress hos mikrobiella nedbrytare observerades också på en större biogeografisk skala inom Europa, trots olika strukturella effekter på de enskilda studieplatserna. Dessutom orsakade fungicider indirekta effekter på detritivorer genom att minska lövmassans smaklighet och påverka detritivorernas födoval. Vidare ledde strukturella förändringar på den mikrobiella nivån till att lövförnans födokvalitet minskade för detritivorer. Vid födointag av lövförna med lägre kvalitet påverkades detritivorernas bearbetning av födan (konsumtion och utsöndring) och resulterade i lägre lipidhalt och tillväxt. Samma typ av påverkan hittades när detritivorer exponerades direkt via vatten, men där var effekterna ännu mer uttalade. Ingen av dessa effektvägar bör emellertid ignoreras med tanke på den additiva effekt de har på detritivorerna.

Sammanfattningsvis kan risker för energiförsörjningen de lägre trofinivåerna i akvatiska näringsvävar förväntas ute i fält vid exponering för fungicider, eftersom effekter på nedbrytare och detritivorer observerades i naturen observerade koncentrationer. Koncentrationerna kan även förväntas öka i framtiden på grund av jordbrukets intensifiering. Detta är oroande, med tanke på den centrala roll detritivorer spelar vid överföring av energi från primärproducenter och lövförna till högre trofinivåer och den risk för dominoeffekter längs näringskedjan som kan följa en sådan störning.

Nyckelord: Bakterier, Biofilm, Detritivorer, Detritus, Direkta effekter, Interaktioner mellan trofinivåer, Lövnedbrytning, Svampar

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Dedication

To curiosity.

If we're gonna survive on this planet, we have to respect the rights of all those species to survive. 'Cause we need them more than they need us.

Tom Searle, Sam Carter, Alex Dean, Dan Searle
(Architects – The Devil Is Near)

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Feckler, A.**, Kahlert, M., Bundschuh, M. (2015). Impacts of contaminants on the ecological role of lotic biofilms. *Bulletin of Environmental Contamination and Toxicology*, 95, pp. 421-427.

II **Feckler, A.**, Goedkoop, W., Konschak, M., Bundschuh, R., Kenngott, K.G.J., Schulz, R., Zubrod, J.P., Bundschuh, M. (in press). History matters: heterotrophic microbial community structure and function adapt to multiple stressors. *Global Change Biology*. DOI: 10.1111/gcb.13859

III Schreiner, V.C., **Feckler, A.**, Fernández, D., Frisch, K., Muñoz, K., Szöcs, E., Zubrod, J.P., Bundschuh, M., Rasmussen, J.J., Kefford, B.J., Axelsen, J., Cedergreen, N., Schäfer, R.B. (Manuscript). Converging ecosystem functioning under stress across biogeographical regions.

IV Zubrod, J.P., Englert, D., **Feckler, A.**, Koksharova, N., Konschak, M., Bundschuh, R., Schnetzer, N., Englert, K., Schulz, R., Bundschuh, M. (2015) Does the current fungicide risk assessment provide sufficient protection for key drivers in aquatic ecosystem functioning? *Environmental Science and Technology*, 49, pp. 1173-1181.

V **Feckler, A.**, Goedkoop, W., Zubrod, J.P., Schulz, R., Bundschuh, M. (2016) Exposure pathway-dependent effects of the fungicide epoxiconazole on a decomposer-detritivore system. *Science of the Total Environment*, 571, pp. 992-1000.

Papers I, II, IV, and V are reproduced with the permission of the publishers.

The contribution of Alexander Feckler (AF) to the papers included in this thesis was as follows:

I AF had the main responsibility of compiling the literature and writing of the manuscript with support of all authors.

II AF had the main responsibility during design and performance of the experiment, analyses of the data, and writing the manuscript with support of all authors.

III AF had the main responsibility for performing the experiment in Sweden and contributed to the analyses of the data. VS and AF had the main responsibility for writing the manuscript with support of all authors.

IV JPZ had the main responsibility during the study, AF contributed to the experiment and analyses of the data. All authors contributed to the writing of the manuscript.

V AF had the main responsibility during design and performance of the experiment, analyses of the data, and writing the manuscript with support of all authors.

Abbreviations

ANOVA	Analysis of variance
C	Carbon
FA	Fatty acid
NMDS	Non-metric multidimensional scaling
NO_3-N	Nitrate nitrogen, i.e. the molecular weight of N in nitrate
PERMANOVA	Permutational multivariate analysis of variance
PUFA	Polyunsaturated fatty acid
RDA	Redundancy analysis

1 Introduction

Mankind has profoundly altered the global environment by influencing land use, biogeochemical cycles, and biodiversity (Crutzen, 2002; Vitousek *et al.*, 1997), despite only being present on a minute geological time scale of Earth's history (Lewis & Maslin, 2015; Zalasiewicz *et al.*, 2011). The environmental conditions of rivers and streams, as well as their ecosystem structure and function, are impacted by a multitude of stressors such as water abstraction for irrigation (Malmqvist & Rundle, 2002), channelisation to facilitate drainage (Blann *et al.*, 2009), increased suspended particle transport to surface waters (Bakker *et al.*, 2008), and water pollution by pesticides and fertilisers (MEA - Millennium Ecosystem Assessment, 2005). These stressors result from agricultural land-use, which already covers one third of the world land area (Bringezu *et al.*, 2014), and the associated land-use changes. Current human population growth, however, will lead to a doubling of the global food demand until 2050 (Godfray *et al.*, 2010) and the necessary agricultural expansion will induce further changes in land-use, thus putting pressure on still unpolluted ecosystems (World Wildlife Fund - Australia, 2015; Laurance *et al.*, 2014). Up to one billion hectares more of natural ecosystems is predicted to be converted to agriculture by 2050, which is accompanied by a 2.4- to 2.7-fold increase in fertiliser and pesticide (e.g. fungicide) contamination in these ecosystems (Tilman *et al.*, 2001). In addition, the predicted global change in temperature and humidity patterns (Intergovernmental Panel on Climate Change, 2013) will allow plant-pathogenic fungi to advance to higher latitudes and further increase fungicide use in order to secure crop harvests (Fisher *et al.*, 2012; Hakala *et al.*, 2011).

In modern agriculture, the application of fungicides is seen as one of several essential measures to secure global food supply, since the proportion of crops lost to plant-pathogenic fungi could feed more than 8% of the world human population (Fisher *et al.*, 2012; Strange & Scott, 2005). Fertiliser application, on the other hand, provides agricultural soils with nutrients (mainly nitrogen, phosphorus, and potassium) necessary to improve crop yields (Roberts, 2009). During or following their application, however, fungicides and fertilisers simultaneously enter adjacent surface waters *via*, for instance, surface run-off (Ulén *et al.*, 2012; Schulz, 2004; Sharpley *et al.*, 2001). In addition, leaching of fertilisers from agricultural soils (Sebilo *et al.*, 2013) contributes to their co-occurrence with fungicides in surface waters. In the receiving streams, a large range of fungicides is commonly detected in mixtures (Fernández *et al.*, 2015; Bereswill *et al.*, 2012; Battaglin *et al.*, 2011; Süss *et al.*, 2006) due to the prophylactic treatment with up to 10 applications of different fungicides per growing season and the multitude of active ingredients used (>150 fungicide active ingredients approved for application in the EU; EC - European Commission, 2017). Ultimately, the exposure to chemical stressors, such as fungicides and fertilisers, contributes to shifts in aquatic community structure and ecosystem processes as for instance detritus decomposition (Fernández *et al.*, 2015; Malaj *et al.*, 2014; Schäfer, 2012).

Small, low-order streams in forested catchments are strongly dependent on allochthonous organic material, particularly leaf litter, to supply local and downstream food webs with energy and nutrients (Webster, 2007; Wallace & Eggert, 1997). Although leaf litter itself offers a lower nutritional quality than autochthonous primary producers, this terrestrial subsidy fuels in-stream microbial respiration and is of central importance for invertebrate secondary production (Marcarelli *et al.*, 2011). Before the leaf-litter-stored energy becomes available for the majority of aquatic organisms, it has to be processed by microbial decomposers and leaf-shredding, detritivorous macroinvertebrates (Fig. 1; Gessner *et al.*, 2007; Cummins & Klug, 1979). In this process, microorganisms (especially the polyphyletic group of aquatic hyphomycetes; Gessner *et al.*, 2007) fulfil two fundamental functions (Suberkropp & Klug, 1976). On one hand, a substantial share of the microbial leaf litter decomposition is accomplished by aquatic hyphomycetes (Taylor & Chauvet, 2014; Hieber & Gessner, 2002). On the other hand, the microbial colonisation conditions the leaf litter for leaf-shredding detritivores by increasing the leaves' nutrient content (e.g. lipids and proteins) and degrading more recalcitrant leaf components (Bärlocher, 1985). Such transformations modify the low-nutritional leaves into a more palatable and nutritious food source for leaf-shredders (Graça *et al.*, 1993a). By their processing of leaf litter, detritivores serve as a key link in detrital food webs as they produce fine particulate organic matter (faecal pellets and leaf fragments) that is used as a food source by collecting macroinvertebrate species (Fig. 1; Cummins & Klug, 1979).

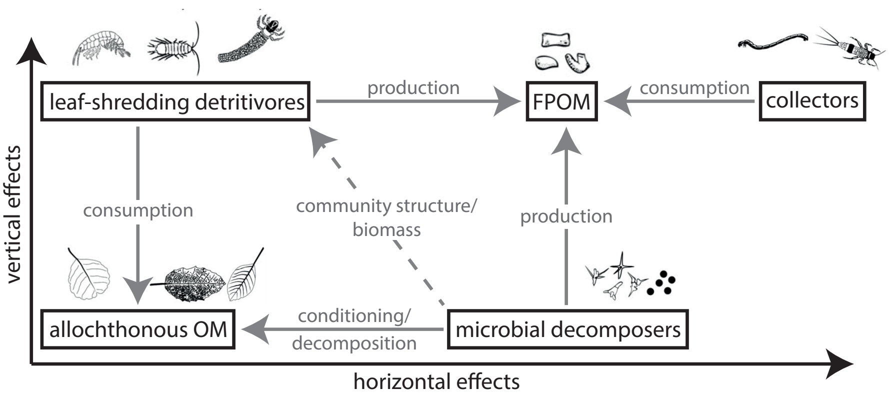

Figure 1. Conceptual scheme illustrating "horizontal" and "vertical" interactions in detrital food webs. The energy input includes allochthonous organic matter (OM) from the adjacent riparian vegetation. Dissolved and fine particulate organic matter (FPOM) originating from external sources and upstream reaches are excluded. Different functional groups of organisms contribute to the transfer of energy through the food web. Arrows represent the dominant processes and parameters that may be affected by stressors with effects on local and downstream aquatic communities. Continuous and dashed arrows indicate direct and indirect effect pathways, respectively (drawings from Bundschuh & Mckie, 2015; Feckler *et al.*, 2015).

At the same time, leaf-shredders are an important prey for aquatic and terrestrial predators (MacNeil *et al.*, 1999). The bottom-up regulation of food webs in detritus-based stream ecosystems highlights the importance of decomposer-detritivore interactions for nutrient and energy cycling from local to larger scales (Wallace & Eggert, 1997).

Anthropogenic stressors like fungicides and nutrients (i.e. fertilisers) can, however, affect the ecological integrity of microbial decomposers and detritivores (Fig. 1; e.g. Zubrod *et al.*, 2017b; Hunting *et al.*, 2016; Zubrod *et al.*, 2015b; Zubrod *et al.*, 2015c; Dimitrov *et al.*, 2014). Fungicides are applied to suppress plant-pathogenic fungi, while non-target fungi such as aquatic hyphomycetes may be affected collaterally. Indeed, several recent studies under laboratory (Dimitrov *et al.*, 2014; Flores *et al.*, 2014; Artigas *et al.*, 2012; Bundschuh *et al.*, 2011; Dijksterhuis *et al.*, 2011; Zubrod *et al.*, 2011) and field conditions (Fernández *et al.*, 2015) have revealed adverse fungicide effects on the structure and function of aquatic hyphomycetes in the µg/L range when exposed through the water. By contrast, nutrient enrichment (in particular nitrate nitrogen (NO_3-N); Fernandes *et al.*, 2014) positively affects aquatic hyphomycetes' structure and activity, and consequently stimulates microbial functioning (Ferreira & Chauvet, 2011; Gulis & Suberkropp, 2003). However, the extent of functional effects caused by structural changes may depend on the diversity of the undisturbed microbial community and the tolerance of species. According to the redundancy hypothesis, more diverse communities display a higher functional stability under stress than communities with a low diversity (Naeem & Li, 1997). The redundancy hypothesis might be explained by the greater chance to find tolerant species in more diverse communities, which can compensate for the loss of more sensitive competitors (sampling effect; Huston, 1997). To put it differently, microbial communities with an exposure history to chemical pollutants might be more resistant to stressor(s) due to a previous replacement of sensitive species by more tolerant ones; a process called pollution-induced community tolerance (Blanck, 2002).

In contrast to microbes, leaf-shredding detritivores can be affected by chemical stressors both *via* the water phase and *via* their food, whereas those pathways' combination can be assumed as a realistic worst-case scenario (cf. Wilding & Maltby, 2006). Many fungicides target physiological processes that regulate energy production and cell division, which are not specific to fungi (Stenersen, 2004). Consequently, fungicides may affect a wide range of aquatic organisms (Maltby *et al.*, 2009), including leaf-shredding detritivores. Functional effects on detritivores, for example a reduction in their feeding activity, have already been demonstrated for several species of the leaf-shredding amphipod genus *Gammarus*, when exposed to individual fungicides and their mixtures directly through the water (e.g. Flores *et al.*, 2014; Zubrod *et al.*, 2014). Moreover, fungicides can affect leaf-shredders via their diet (Fig. 1). First, fungicides may cause toxic effects when sorbed to leaf material (Dimitrov *et al.*, 2014) and thus are co-ingested during consumption, as reported for other pesticides (Bundschuh *et al.*, 2013). Second, since fungicides can negatively affect fungal decomposers, the microbial conditioning process of leaves is hampered which consequently reduces their palatability and food quality for shredders (Bundschuh *et al.*, 2011). Besides reductions in fungal biomass, aquatic hyphomycete community composition can also explain adverse effects on leaf-shredders due to their reported selective feeding (Bundschuh *et al.*, 2011; Arsuffi & Suberkropp, 1989). Effects on leaf colonisation by bacteria and fungi can therefore indirectly affect the physiology and life history strategies of leaf-shredders with subsequent effects on ecosystem functioning (Zubrod *et al.*, 2017b; Zubrod *et al.*, 2015b).

2 Objectives and research questions

Our knowledge of fungicide effects on organisms and processes mediating leaf litter decomposition as well as effects on the trophic relationships at the base of the food web is still scarce, despite a growing number of studies over the past years. The primary objective of this thesis was therefore to contribute with additional knowledge on the eco(toxico)logical effects of fungicides on microbial decomposers and primary consumers. The synthesis of the current scientific knowledge on fungicide effects on microbial decomposers and leaf-shredding detritivores in Paper I allowed me to identify several knowledge gaps. For example, few studies to date have investigated the influence of chemical stress exposure history on fungicide effects on leaf-associated microbial decomposers. Moreover, we are just beginning to understand the propagation of effects on microbes to higher trophic levels and how the strength of such indirect effects differs from waterborne fungicide toxicity to detritivores. These knowledge gaps were experimentally addressed in this thesis with the following underlying research questions:

1. Does an exposure history to pesticides and nutrient enrichment influence structural and functional effects on decomposers? (Paper II)
2. Does the response of decomposers from unpolluted streams to stress converge across biogeographical regions with time? (Paper III)
3. Do environmentally relevant fungicide concentrations induce indirect bottom-up effects on key leaf-shredders by altering leaf litter-associated microbial communities? (Paper IV)
4. What is the relative importance of food-associated and waterborne fungicide exposure for leaf-shredding detritivores that exhibit a conjectured higher tolerance to nutrient stress? (Paper V)

3 Materials and methods

3.1 General design of the experimental studies

In **Paper II,** I studied how a chemical stress exposure history can modulate waterborne toxicity of fungicides to decomposing microbes. The microbial inoculum (see part 3.2) used during the experiment originated from streams situated either in a forested catchment (=unpolluted) or one with a high share of agricultural land-use (step I in Fig. 2). Differences in the exposure history of the microbial communities were conjectured based on the differences in catchment land-use and in their pesticide and nutrient loads. Afterwards, this inoculum was applied to microbially colonise fresh leaf material under exposure to a fungicide mixture (azoxystrobin, carbendazim, cyprodinil, quinoxifen, and tebuconazole; Table 1) and various levels of nutrients for 21 days in a full-factorial, semi-static design (n=6; step II in Fig. 2). The microbial leaf decomposition rate was quantified as a functional variable (Benfield, 2007). To facilitate a mechanistic understanding of the observed functional effects, several structural variables were measured (step III in Fig. 2): leaf-associated ergosterol content was quantified as a proxy for fungal biomass (Gessner, 2005) and the aquatic hyphomycete community composition was determined by their spore morphology (Fernandes *et al.*, 2014; Bärlocher, 1982).

I hypothesised that fungicides affect microbial leaf decomposition rates, while nutrients compensate for these implications by stimulating microbial activity. In addition, the functional responses should be explained by structural alterations of the leaf-associated microbial community, namely changes in the aquatic hyphomycete community composition, number of released conidia, and fungal biomass. Finally, I hypothesised that the strength of the effects depends on the exposure history, with less pronounced effects after previous exposure in the field.

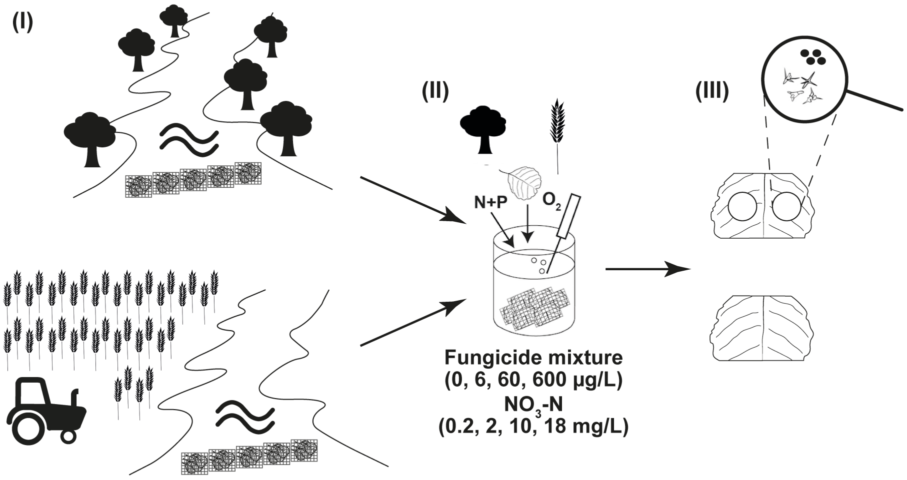

Figure 2. Schematic illustration detailing the experimental design of Paper II: (I) pre-conditioning of fresh leaf material in streams situated either a forested (trees) or agriculturally used (cropland) catchment; (II) microbial colonisation of fresh leaf material with microbial inoculum originating from one of the pre-conditioning sites under exposure to a fungicide mixture and varying nutrient levels; (III) quantification of microbial leaf decomposition and analyses of leaf-associated microbes. NO_3-N is reported as the nutrient with the highest effect on microbial leaf decomposition (Fernandes *et al.*, 2014).

In **Paper III**, we studied if the functional and structural responses of decomposing microbes to fungicides show a similar pattern across on a larger biogeographical scale (=convergence). Therefore, the study was conducted in the Central Plains (Denmark), the Western Highlands (Germany), and the Fenno-Scandian Shield (Sweden), over seven weeks following the same protocol. Briefly, three consecutive sets of leaf material were used corresponding to three microbial colonisation cycles (Fig. 3; depicted in distinct colours). The first leaf set was microbially colonised for 7 days in one unpolluted stream per region, upstream of any urban or agricultural influence (step I in Fig. 3). Afterwards, colonised leaves were directly introduced into the test system (n = 7 in Denmark and Germany; n = 6 in Sweden; step II in Fig. 3). The subsequent leaf sets were colonised by the leaf-associated microbial community present on the previous leaf sets (steps III and V in Fig. 3). At the end of each colonisation cycle (i.e. at days 21, 35, and 49), microbial leaf decomposition was quantified as a functional variable, whereas structural changes were characterised by the community composition and sporulation of aquatic hyphomycetes (step IV in Fig. 3). With this setup, adaptations of the aquatic hyphomycete communities under fungicide stress were captured over the three consecutive colonisation cycles. Vegetative growth and reproduction both contribute to aquatic hyphomycete community dynamics, where reproduction is typically triggered through resource (leaf) availability (Bärlocher, 1992). Our setup allowed us to track effects on reproduction since sporulation, as one pathway for the colonisation of new resources, is usually more sensitive to stress than vegetative growth (Lecerf & Chauvet, 2008).

During the laboratory experiments, the microcosms were subjected to two treatments: a fungicide-free control and a fungicide mixture (metalaxyl, prothioconazole, pyrimethanil; Table 1). The fungicide mixture was applied in short-termed episodic peaks (2 days) mimicking the upper end of fungicide toxicity concentrations observed in a meta-analysis of global pesticide levels (i.e. sumTU-1; Schäfer *et al.*, 2012). Peaks were interspersed with continuous exposures (12 days) at 10-fold lower fungicide concentrations (i.e. sumTU-2) to simulate pesticide drainage within catchments (Bundschuh *et al.*, 2014; upper panel in Fig. 3).

We hypothesised that fungicides should negatively affect microbial leaf decomposition, while these effects should be explained by changes in the aquatic hyphomycete sporulation and community composition. Moreover, we hypothesised that changes in the aquatic hyphomycete composition should lead to an adaptation of communities to fungicide stress enabling a stable or even increased leaf decomposition towards the end of the experiment.

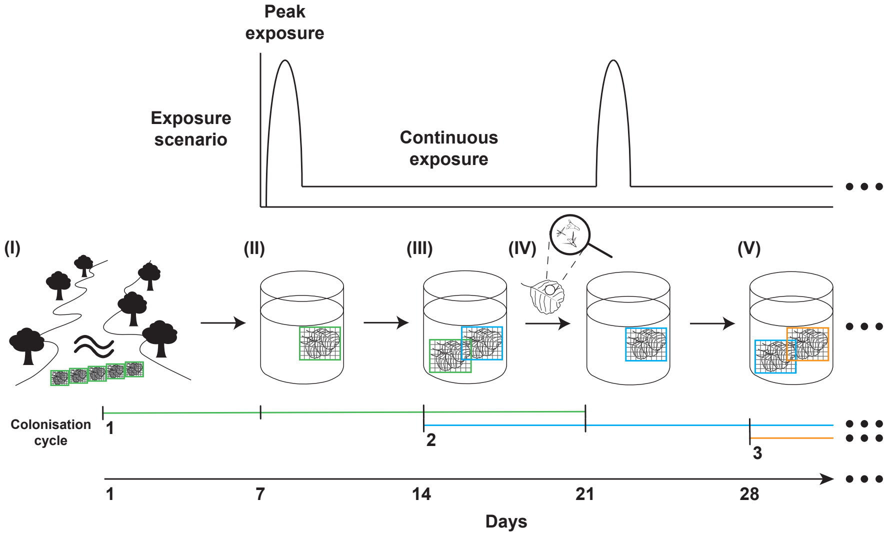

Figure 3. Schematic illustration detailing the experimental design of Paper III: (I) conditioning of the first leaf set in unpolluted streams; (II) introduction of the first leaf set into the test system; (III & V) conditioning of the second and third leaf set in the test system (7 days; overlapping bars) and retrieval of the first and second leaf set thereafter; (IV) quantification of leaf decomposition and cutting of leaf discs for analyses of leaf-associated microbes. Fungicides were applied as short-termed episodic peaks, interspersed with continuous exposures at 10-fold lower concentrations (upper panel).

In **Paper IV**, we studied the waterborne toxicity of fungicides to decomposing microbes and the effect propagation to leaf-shredding detritivores. Therefore, microbial inoculum originating from an unpolluted stream (step I in Fig. 4) was used to colonise leaf discs under control and treatment conditions, namely exposed to the fungicides used during Paper II, for 12 days (step II in Fig. 4). Afterwards, microbial decomposition as well as the palatability of leaf material for leaf-shredding detritivores were quantified. For the latter, the colonised leaf material was fed to the leaf-shredder *Gammarus fossarum* in a food choice experiment (cf. Bundschuh *et al.*, 2011): one leaf disc each from one of the fungicide treatments and the control were simultaneously offered to the test organisms and *Gammarus'* food choice was observed (*n*=49; step IV in Fig. 4). The same set of structural variables as described for Paper II was determined for a mechanistic understanding of the observed functional effects (*n*=7; step III in Fig. 4). In addition, leaf-associated bacterial abundance was assessed using epifluorescence microscopy (Büsing, 2005).

Moreover, the potential repellent effect of leaf-adsorbed fungicides on the food choice of *Gammarus* (Rasmussen *et al.*, 2012; Hahn & Schulz, 2007) was studied following a similar experimental design. During these additional experiments, pre-conditioned but autoclaved leaf discs were used to prevent stimulating or repellent effects of the microbial community. The effect of leaf-adsorbed fungicides on the food choice of *G. fossarum* was simulated for the lowest fungicide concentration for which a significant effect was found in the main experiment (or the highest concentration if no significant effect was found). For both types of experiments, fungicides were first tested individually and then as a mixture at a fixed concentration ratio (Jonker *et al.*, 2011).

We hypothesised that fungicides alter the structure of the leaf-associated microbial community, namely changing the aquatic hyphomycete community composition, fungal biomass and bacterial abundance. These structural changes were hypothesised to induce changes in the microbial leaf decomposition and the food choice of *G. fossarum*, given its selective feeding behaviour on fungi (Arsuffi & Suberkropp, 1989). Finally, leaf-adsorbed fungicides were hypothesised to contribute to the food choice of *G. fossarum*.

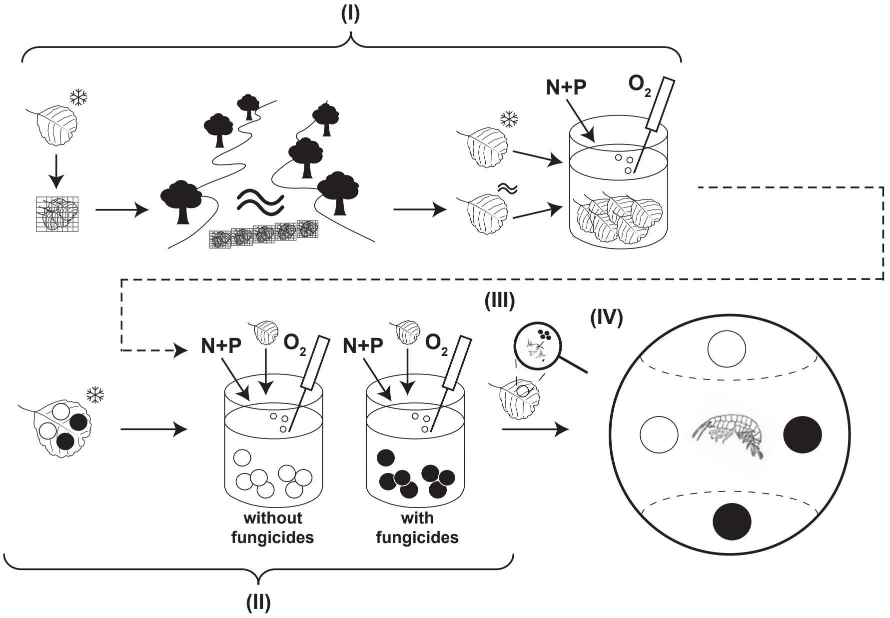

Figure 4. Schematic illustration detailing the experimental design of Paper IV: (I) generating inoculum using a microbial community from an unpolluted stream; (II) conditioning of leaf discs in absence and presence of fungicides; (III) quantification of microbial leaf decomposition and analyses of leaf-associated microbes; (IV) food-choice experiment with *G. fossarum* (adapted after Zubrod *et al.*, 2015c).

In **Paper V**, I applied a full 2×2-factorial design (cf. Zubrod *et al.*, 2015b) to disentangle the relative importance of the food-associated and the waterborne fungicide toxicity for leaf-shredding detritivores. The first factor was the absence or presence of a fungicide (epoxiconazole; Table 1) during the 10 days of microbial colonisation of leaf material that was used as food during the experiment (step IV in Fig. 5). The second factor was the absence or presence of the same fungicide in the test medium during a 28-day experiment, where *Asellus aquaticus* was fed with the leaf material described beforehand (*n*=40; step VI in Fig. 5).

A. aquaticus was chosen as test species for Paper V, since food-associated fungicide effects have only been studied for *Gammarus* species within the diverse functional group of shredders. Although the abundance of *Gammarus* can be high in undisturbed stream sections, abundance can be relatively low in streams affected by nutrient pollution (e.g. agricultural streams; Whitehurst, 1991). In such impacted streams, *A. aquaticus* often becomes the predominant shredder and takes over the function typically attributed to *Gammarus* (Graça *et al.*, 1993b).

Fungal biomass, bacterial abundance, the microbial fatty acid (FA)-profile (Arce Funck *et al.*, 2015), and the capacity of leaf-associated microorganisms to metabolise different carbon (C)-substrates (using EcoPlates^{TM}; Maul *et al.*, 2006) were quantified to understand effects on microbial communities and the food quality of the leaf material for *Asellus* (step V in Fig. 5). Furthermore, the effects of the distinct pathways and their combination on *Asellus* were judged by its food processing behaviour (leaf consumption and excretion; e.g. Maltby *et al.*, 2000) and the physiological fitness of *Asellus* in terms of growth, lipid content (Van Handel, 1985), and FA-profile (Lau *et al.*, 2012).

I hypothesised that epoxiconazole modifies the leaf-associated microbial community, in terms of changes in the fungal biomass, bacterial abundance, FA-profile, and metabolisation of different C-substrates. The structural changes in fungal biomass and the microbial FA-profile should ultimately affect the food processing behaviour and physiology of *Asellus* when feeding on the fungicide-affected leaf material. Additionally, I hypothesised direct effects of epoxiconazole on *Asellus* through the water phase. Based on the results of earlier studies (e.g. Zubrod *et al.*, 2015b), I further assumed that the effect pathways cause additive effects when applied in combination.

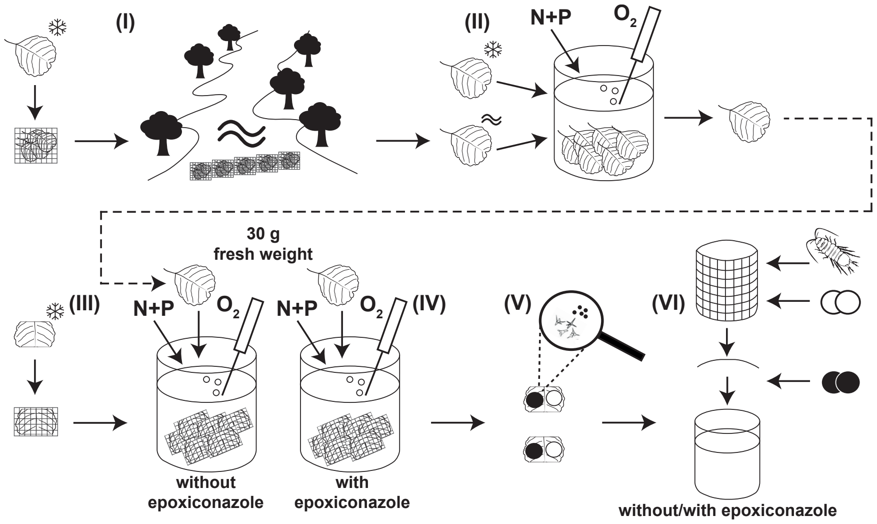

Figure 5. Schematic illustration detailing the experimental design of Paper V: (I) pre-conditioning of fresh leaf material in an unpolluted stream; (II) pre-conditioned and fresh leaf material kept in nutrient medium to generate microbial inoculum for the experiment; (III) cutting leaf strips from fresh leaf material and packing them into nylon fine-mesh bags; (IV) conditioning of leaf strips by microbial inoculum in absence and presence of epoxiconazole; (V) cutting of leaf discs used as food for the experiment and analyses of leaf-associated microbes; (VI) experiment to disentangle the relevance of food-associated and waterborne toxicity of epoxiconazole (Feckler *et al.*, 2016).

3.2 Sources of leaf material, microbial decomposers and detritivores

Senescent but undecomposed black alder leaves (*Alnus glutinosa* (L.) Gaertn.) were collected from trees near Landau, Germany (e.g. N 49°12'; E 8°13'), during autumn and stored at -20 °C. Leaf material was packed into fine-mesh leaf bags (0.5 mm mesh size) and deployed in streams to obtain leaf-associated microbial communities. The complete descriptions of the sampling sites for microbial communities can be found in the individual papers. Bags were retrieved from the streams after 14 days (except for Paper III; see above) and the leaf material was carefully cleaned under running water to remove macroinvertebrates. Next, fresh (=unconditioned) leaf material was added and kept for another 14 days to generate inoculum that features microbial communities at various stages of colonisation (Gessner *et al.*, 2003). The usage of diverse natural leaf-associated microbial communities was favoured over conducting experiments with a defined community of few aquatic hyphomycete species reared in the laboratory (e.g. Ferreira & Chauvet, 2011). By this procedure, all microbial species interactions (i.e. between fungi and bacteria) that may influence the structural and functional effects of fungicides in these communities were taken into account (Brandt *et al.*, 2015).

Two leaf-shredding detritivore species were used as model organisms. First, *Asellus aquaticus* (L.) (Crustacea: Isopoda; Paper V), sampled in a shallow pond in Uppsala, Sweden (N 59°48'; E 17°39'), was selected due to its conjectured higher tolerance to nutrient pollution than *Gammarus* (Whitehurst, 1991), but well-documented sensitivity to pesticides (e.g. Bundschuh *et al.*, 2012; Beketov & Liess, 2008). Second, *G. fossarum* KOCH (Crustacea: Amphipoda; cryptic lineage B; Feckler *et al.*, 2014; Paper IV), sampled at the Hainbach, Germany (49°14'N; 8°03'E), was used because of its key relevance in the process of leaf litter decomposition (Englert *et al.*, 2013; Dangles *et al.*, 2004), its sensitivity to chemical stressors (e.g., Zubrod *et al.*, 2014), and well-documented food choice behaviour (Bundschuh *et al.*, 2011; Arsuffi & Suberkropp, 1989). Leaf-shredders were sampled at least one week prior to the experiments to allow acclimation to laboratory conditions and the test medium.

3.3 Chemicals

Nine current-use organic fungicides were selected that cover a broad range of modes of toxic action. Fungicides were either applied in the form of commercially available pesticide formulations or using analytical standards (Table 1). Furthermore, NO_3-N and phosphate-phosphorus were used in nutrient additions in Paper II, since higher concentrations in surface waters are expected from future agriculture (Roberts, 2009). The nominal concentrations of fungicides and nutrients were validated using an ultrahigh-performance liquid chromatography-mass spectrometry system and an automated photometric analyser (both Thermo Fisher Scientific, Dreieich, Germany), respectively. Detailed descriptions of the methods are given in the respective papers.

Table 1. *Fungicides used during the present thesis with information on their respective chemical family, mode of toxic action, and papers of the thesis where the individual fungicides were used.*

Fungicide	Applied product	Chemical family	Mode of toxic action[a]	Used in paper(s)
Azoxystrobin	Ortiva®	Strobulins	Mitochondrial respiration inhibitors	II & IV
Carbendazim	Derosal©	Benzimidazoles	Mitosis and cell division inhibitors	II & IV
Cyprodinil	Chorus®	Anilinopyrimidines	Amino acid and protein synthesis inhibitors	II & IV
Epoxiconazole	Opus®	Triazoles	Sterol biosynthesis inhibitors	V
Metalaxyl	Analytical standard	Acylalanines	Fungal nucleic acid synthesis disruptors	III
Tebuconazole	Folicur®	Triazoles	Sterol biosynthesis inhibitors	II & IV
Prothioconazole	Analytical standard	Triazolinthione	Sterol biosynthesis inhibitors	III
Pyrimethanil	Analytical standard	Anilino-Pyrimidines	Methionine protein synthesis inhibitors	III
Quinoxyfen	Fortress® 250	Quinolines	Signal transduction perturbators	II & IV

[a] Fungicide Resistance Action Committee (2017)

3.4 Statistical analyses

The open source statistical software R (versions 3.0.2 – 3.3.3 for Mac OS X; R Development Core Team, 2017), supplemented by the required add-on packages, was used to conduct all statistical analyses and prepare figures. The term “significant(ly)” is hereafter exclusively used in the sense of “statistical significance”. Comprehensive descriptions of the applied statistical methods can be found in the individual papers.

Depending on the test design, either one- (Paper IV), two- (Papers III & V) or three-way ANOVAs (Paper II) were performed on univariate data (all variables with exception of the C-metabolisation, aquatic hyphomycete community composition and FA-profiles) to determine the significance of the assessed factors and their interplay. A graphical assessment of potential interactive effects of the applied chemicals was performed by multiple linear regressions that were visualised as two-dimensional surface plots displaying the fitted response values against a surface defined by the two stressors (Paper II; Feld *et al.*, 2016). In addition, *t*-tests were performed if only two sets of data (control and one treatment) were compared. In cases of non-normality of residuals or homoscedasticity, Wilcoxon signed-rank tests and Kruskal−Wallis tests were applied as non-parametric alternatives (for all univariate analyses see Zar, 2010).

Furthermore, several models (including lognormal, log-logistic, Weibull, Cedergreen-Ritz-Streibig, and Michaelis-Menten models) were fitted to the data to either assess the response of the microbial decomposition rate to the tested fungicide concentrations (Paper II) or to assess the microbial C-metabolisation over time (Paper V). The models fitting the data best were selected by visual judgment and Akaike’s information criterion. Confidence interval testing (Wheeler *et al.*, 2006) was used to assess significant differences in the microbial decomposition rate between the assessed communities (Paper II) or in the C-metabolisation between the control and the fungicide treatment (Paper V). Finally, the joint effects of the five fungicides applied during the mixture experiment (Paper IV) were judged by comparing the observed effect sizes to the predictions of the “independent action” model that assumes dissimilar modes of toxic action among the mixture components (Bliss, 1939). The applicability of the “independent action” model on the community level has been shown by Backhaus et al. (2004).

For multivariate data on aquatic hyphomycete community composition and FA-profiles, shifts compared to the fungicide-free controls were identified using permutational multivariate analyses of variances (PERMANOVAs).

Non-metric multidimensional scaling (NMDS) ordination plots (Clarke, 1993) were prepared for a graphical interpretation of the observed shifts. Stress values were calculated as a measure of “goodness-of-fit” for NMDS, with values below 0.2 showing a reasonable fit of the ordination (Clarke, 1993). Alternatively (Paper III), redundancy analyses (RDAs) followed by permutational type III ANOVAs (Legendre *et al.*, 2011) were conducted.

The discussions of the individual papers are, however, not exclusively based on the significance of the results. The reason is that the application and interpretation of null hypothesis significance testing are heavily debated (Nakagawa & Cuthill, 2007). Null hypothesis significance testing does not determine the magnitude of an observed effect (i.e. the effect size) and thus may deflect attention from its biological relevance (European Food Safety Authority, 2011), while *P*-values and biological relevance are not necessarily linked (e.g. Burnham & Anderson, 2014; Nuzzo, 2014). Hence, a relative effect size that expresses the percentage change compared to the respective control treatment (Kaiser, 1989) is provided irrespective of significance wherever mathematically calculable.

4 Results and Discussion

4.1 The role of exposure history for structural and functional effects on decomposers (Paper II)

As hypothesised, fungicide exposure affected microbial leaf decomposition, while the effect pattern was dependent on the microbial communities' exposure history, as indicated by a significant two-way interaction between the factors "fungicide" and "exposure history" ($P = 0.031$). More specifically, exposure to ≥ 60 µg fungicides/L impaired leaf decomposition by microbes from the unpolluted stream by 25% at the lowest nutrient level (Fig. 6a).

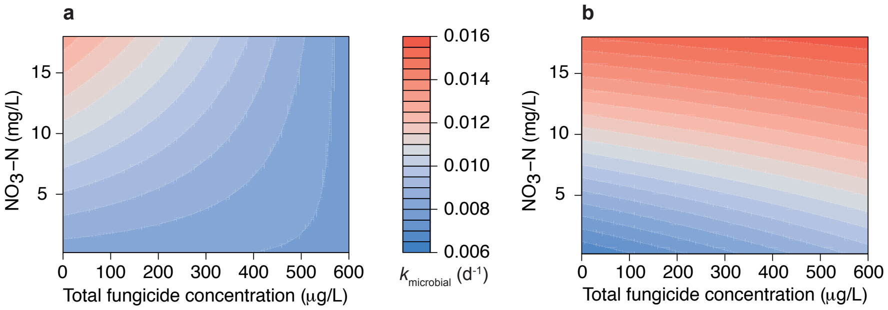

Figure 6. Two-dimensional surface plots visualising the decomposition rates ($k_{\mathrm{microbial}}$) by previously (a) unpolluted and (b) exposed microbial communities against a surface defined by the total fungicide concentration and the NO_3-N concentration (Feckler *et al.*, in press).

In the absence of fungicides, on the other hand, nutrient additions increased the decomposition by microbial communities from the unpolluted stream by 40% across the nutrient gradient (Fig. 6a). In contrast to the expectation, the positive response under nutrient enrichment did not compensate for the negative fungicide effects in previously unpolluted communities. Leaf decomposition was still reduced by ~30% upon fungicide exposure even at the highest nutrient level (Fig. 6a). By contrast, no adverse effects on leaf decomposition were found for the previously exposed community, irrespectively of the fungicide concentration. In fact, the decomposition rate by the previously exposed community was even stimulated especially at low nutrient levels (~85% at the combination of 2.0 mg NO_3-N/L and 600 µg fungicides/L; Fig. 6b). This increase in the functional response upon fungicide exposure is likely explained by the ability of microorganisms to adapt to pesticides and use them as an energy source (e.g. Krauss *et al.*, 2011; Hodkinson & Dalton, 1973). Increased nutrient levels further stimulated the leaf decomposition by the previously exposed community, both in absence (~150%) and presence of fungicides (up to ~90%; Fig. 6b).

The observed discrepancy in leaf decomposition response for the previously unpolluted and exposed communities may be due to distinct structural changes. The history of chemical stress exposure in the field likely allowed for an adaptation to fungicides and enriched nutrient levels in a way that tolerant aquatic hyphomycete species replaced sensitive competitors and maintained the communities' function (cf. Blanck, 2002). This was supported by the observation that the aquatic hyphomycete community composition in controls from the previously exposed stream differed substantially from those from the unpolluted stream under nutrient enrichment. The former communities were dominated by *Tetracladium marchalianum* DE WILDEMAN and *T. setigerum* (GROVE) INGOLD, which were reported to withstand higher fungicide concentrations (e.g. Bundschuh *et al.*, 2011) and decompose leaf litter at a higher rate (Andrade *et al.*, 2016; Raviraja *et al.*, 2006) than other hyphomycete species do. These structural differences are in line with the observed almost fourfold higher increase in the microbial leaf decomposition by the previously exposed than by the unpolluted community under high nutrient availability and suggest a structural adaptation to more eutrophic environments. Moreover, fungicide exposure significantly modified the aquatic hyphomycete composition in both communities (all comparisons $P \leq 0.045$) accompanied by a reduction in species richness. The maintained function despite a lower diversity in the previously exposed community is likely triggered by the dominance of *T. marchalianum* and *T. setigerum*, which both could be proposed as functional drivers under stress. Besides, the mostly unaffected or increased fungal biomass of as well as the unaffected or only marginally reduced number of released conidia by the previously exposed community upon fungicide exposure might have led to increased microbial decomposition rates (Gessner & Chauvet, 1994).

Although it is challenging to draw general conclusions based on a limited number of assessed communities, results suggest that a history of chemical stress exposure can modulate the effect strength, with previously exposed microbial communities being adapted to fungicide stress and more eutrophic environments. This suggests the predicted global large-scale agricultural expansion with increasing uses of fertilisers and fungicides to cause adverse effects on the structure and function of previously unpolluted decomposer communities (Fisher *et al.*, 2012; Hakala *et al.*, 2011; Kattwinkel *et al.*, 2011; Tilman *et al.*, 2001). Moreover, a modification of fungal community structure may result in unpredictable bottom-up directed effects in stream food webs (Gonçalves *et al.*, 2014), given leaf-shredders' selective feeding on aquatic hyphomycetes (Arsuffi & Suberkropp, 1989).

4.2 Stress response of decomposers across biogeographical regions (Paper III)

In Paper III, we observed a convergent dynamic of functional stress response over time across the three biogeographical regions. During the first colonisation cycle, fungicide exposure reduced leaf decomposition significantly by up to some 50% compared to controls ($P \leq 0.033$; Fig. 7).

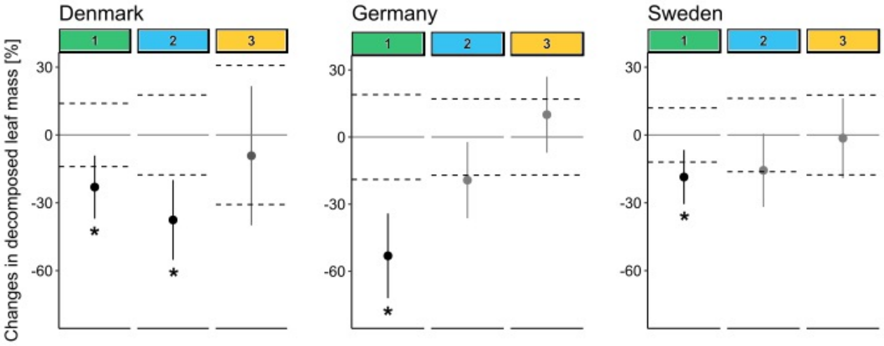

Figure 7. Mean relative changes in decomposed leaf mass (in %; with 95% CIs) in the fungicide treatments compared to the respective controls (solid, horizontal lines; dashed lines indicate corresponding 95% CIs) for the different colonisation cycles (numbers on top; green, blue and orange refer to the first, second and third cycle, respectively). Asterisks denote significant differences to the respective control.

This negative effect of fungicides on leaf decomposition attenuated during the subsequent colonisation cycles and leaf decomposition approached that of the controls during the third cycle in all three regions. Although this temporal pattern was similar across the biogeographical regions, the effect sizes differed at the individual colonisation cycles. For instance, the effect size in Germany during the first colonisation cycle was two-fold higher than those observed in Denmark and Sweden. Moreover, leaf decomposition under fungicide exposure by the microbial communities originating from the German and Swedish streams already increased during the second colonisation cycle and did not significantly differ from that in the controls ($P \geq 0.114$). For the community from the Danish stream, however, this effect remained unchanged during the second colonisation cycle ($P = 0.004$).

The observed initial adverse effects on litter decomposition seem to be a general response of previously unpolluted leaf-associated microbes to organic fungicides that supposedly affect the growth of aquatic hyphomycetes and impair leaf decomposition (Paper II; but see also Rasmussen *et al.*, 2012). Our finding of higher leaf decomposition after three consecutive colonisation cycles (Fig. 7) suggests that the microbial communities had gradually adapted to fungicide stress and ultimately tended to recover to their initial functional performance. Thus, we speculate that a pollution-induced community tolerance was developed during the study (Paper II; but see also Gardeström *et al.*, 2016). Indeed, all hyphomycete communities showed shifts in their composition and species richness, but drivers partially differed among regions. For the microbial communities originating from the Danish and German streams, the structural response was mainly driven by the colonisation cycle with approximately 40% and 15% of explained variance, respectively ($P = 0.001$; Fig. 8). In these regions, fungicide exposure only explained 3% and 6%, respectively, of the variation and was therefore only of minor importance for community composition ($P \geq 0.263$). By contrast, for the microbial community from the Swedish stream, fungicide exposure was the main driver of the observed community changes with 21% explained variance ($P = 0.001$), and with only a minor impact (8%; $P = 0.224$) of the colonisation cycle.

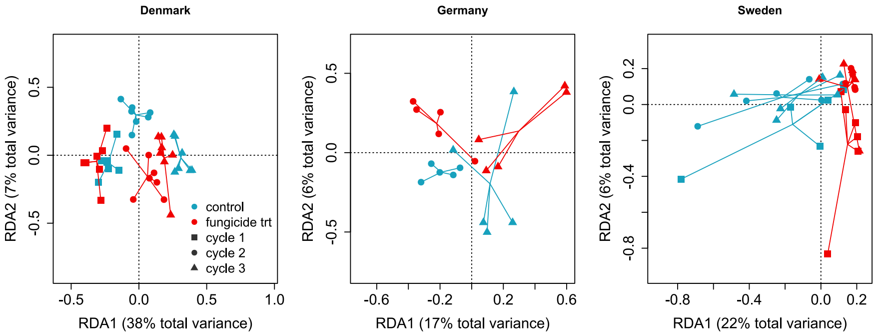

Figure 8. Aquatic hyphomycete compositions of the studied communities. Each point within the RDAs represents the community of one replicate. Replicates of each treatment are connected through lines. Due to technical difficulties, hyphomycete spore identification was not possible for the first colonisation cycle in Germany (see paper for details).

Hyphomycete species richness showed similar tendencies in communities from the Danish and the Swedish stream, with an almost 50% decrease over the colonisation cycles in both the fungicide treatment and the control. This pattern may also apply to the communities from the German stream, but the lack of data from the first colonisation cycle hinders any definite interpretation. Nevertheless, the minor importance of fungicide exposure on the communities from the Danish and German streams is reflected by a relatively similar aquatic hyphomycete species richness and composition across treatments and colonisation cycles (Fig. 8). This suggests that aquatic hyphomycete species that were able to thrive under laboratory conditions also exhibited a higher tolerance to fungicide exposure and maintained decomposition. This induction of tolerance may also apply to the community from the Swedish stream in which the same few species were found in controls and fungicide treatments. However, reduced sporulation of the few aquatic hyphomycete species under fungicide exposure, despite an increase in leaf breakdown, prohibits a more definite interpretation, but explains fungicide exposure being the main driver for the detected structural responses (Fig. 8).

Although it remains speculative to draw conclusions based on only a single stream per region, Paper III suggests that time is an important parameter when assessing convergences in ecosystem functioning across biogeographical scales. As shown here, non-convergence of effects at one point in time (second colonisation cycle) does not exclude convergence at a later stage (third colonisation cycle). Disregarding the temporal dynamics of effects may result in ill-founded management decisions regarding chemical stressor thresholds, which ultimately might result in species losses and impaired ecosystem services. Furthermore, while microbial systems have been used as model systems for eco-evolutionary processes and stress adaptations, the potential to translate the results to higher trophic levels driving important ecosystem functions (e.g. insects or vertebrates) remains open. First, community adaptations to stress at higher trophic levels are likely to occur over longer time periods due to longer generation cycles. Second, since the chemical stress load in surface waters is steadily increasing as a result of agricultural intensification, the time frame needed for adaptation may be exceeded causing exacerbating functional losses.

4.3 Fungicide-induced indirect bottom-up effects on key leaf-shredders (Paper IV)

Microbial leaf decomposition was – as hypothesised – significantly reduced by the fungicides azoxystrobin (~10%; 100 and 500 µg/L; $P \leq 0.011$), carbendazim (~10%; ≥ 245 µg/L; $P < 0.001$), and cyprodinil (~10%; ≥ 200 µg/L; $P \leq 0.001$) compared to controls. Given the reported differences of aquatic hyphomycetes' decomposition rates (e.g. Andrade *et al.*, 2016; Raviraja *et al.*, 2006) changes in their community composition and therefore trait diversity may be seen as the driver for observed effects on decomposition (cf. results in Papers II & III).

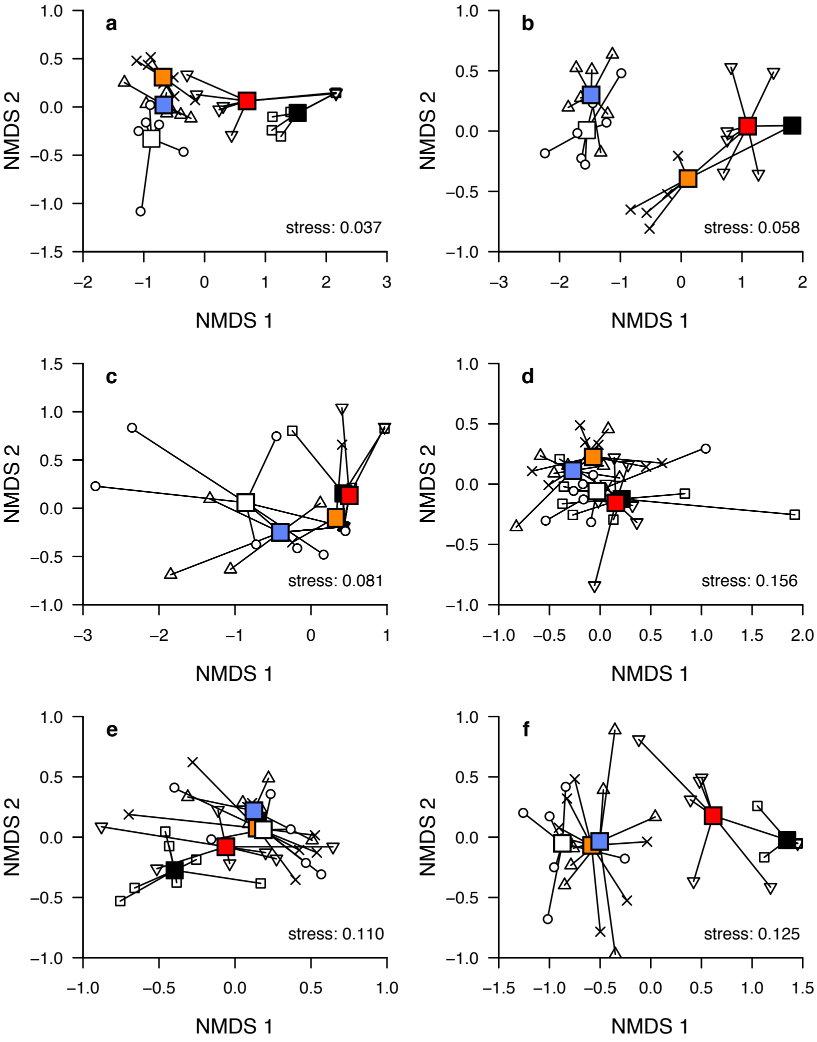

Figure 9. Non-metric multidimensional scaling (NMDS) ordination plots for the aquatic hypomycete communities associated with leaf material conditioned in the presence of (a) azoxystrobin, (b) carbendazim, (c) cyprodinil, (d) quinoxyfen, (e) tebuconazole, and (f) the fungicide mixture. Symbols of the individual samples and the colour scale of their group centroids indicate the fungicide treatments (from lowest to highest): circles/white (control), triangles/blue, crosses/orange, inverted triangles/red, and squares/black (adapted after Zubrod *et al.*, 2015a).

All three fungicides altered fungal communities relative to the controls, with communities being significantly shifted at ≥ 20 µg azoxystrobin/L ($P \leq 0.032$; all coloured group centroids in Fig. 9a) and at ≥ 35 µg carbendazim/L ($P \leq 0.008$; orange, red, and black group centroid in Fig. 9b). Furthermore, non-significant alterations in the community composition at ≥ 40 µg cyprodinil/L ($P \geq 0.112$; orange, red, and black group centroid in Fig. 9c) were accompanied by some 80% reductions in hyphomycete diversity ($P \leq 0.057$), likely explaining the observed functional impairment.

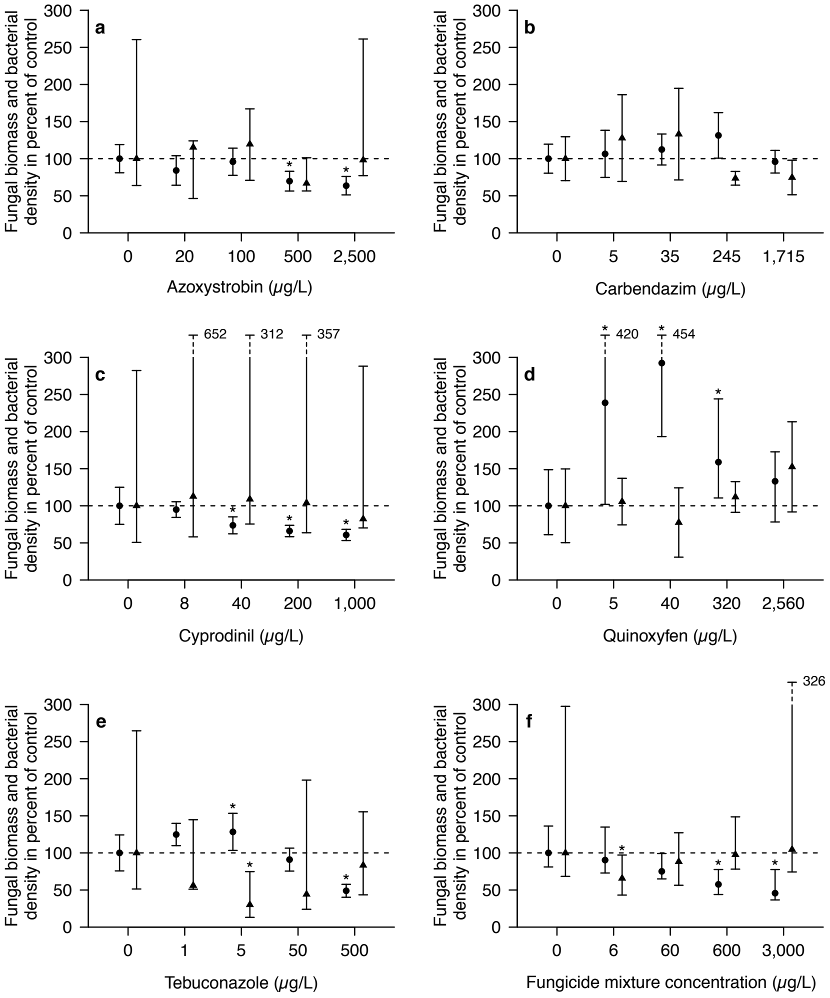

Figure 10. Mean or median (with 95% CI) fungal biomass (circles) and bacterial density (triangles), relative to the respective control, associated with leaf material conditioned in the presence of different concentrations of (a) azoxystrobin, (b) carbendazim, (c) cyprodinil, (d) quinoxyfen, (e) tebuconazole, and (f) the fungicide mixture. Asterisks denote statistically significant differences to the respective control (adapted after Zubrod *et al.*, 2015a).

By contrast, for quinoxyfen and tebuconazole no negative effects on microbial leaf decomposition were found. Interestingly, at 1 µg tebuconazole/L a 10% increase in leaf decomposition compared to the control was observed ($P = 0.028$), suggesting a hormetic effect (Calabrese & Baldwin, 2003). The observed 25% increase in fungal biomass at 1 µg tebuconazole/L ($P = 0.073$), which was likely caused by a decline in competitive pressure on fungi due to a 45% reduced bacterial abundance (Fig. 10e), may have induced the higher leaf decomposition (Gessner & Chauvet, 1994). Although a 30% higher fungal biomass ($P = 0.043$) and 70% reduced bacterial abundance ($P = 0.016$) were consistent also at 5 µg tebuconazole/L, leaf decomposition at this concentration was similar to that of the control. We speculate that this observation may be explained by the ~25% higher reduction in bacterial abundance, given their contribution to leaf decomposition (Hieber & Gessner, 2002). However, also effects on fungal groups other than aquatic hyphomycetes, such as Chytridiomycota, Oomycota, and Zygomycota (cf. Manerkar *et al.*, 2008; Nikolcheva & Bärlocher, 2004), which are likely involved in decomposition but not addressed in our study, may explain this observation.

Besides fungicide-induced effects on microbial leaf decomposition, we observed an effect propagation to leaf-shredding detritivores. This propagation was observed by the significant preference of *Gammarus* for control over fungicide-exposed leaves at the highest concentrations of carbendazim, cyprodinil, quinoxyfen, and tebuconazole ($P \leq 0.005$; Fig. 11b-e). Similar trends were found at lower concentrations of these fungicides and at the highest concentration of azoxystrobin (Fig. 11a). This suggests that all tested fungicides affected leaf palatability negatively. Fungicide exposure resulted in altered leaf-associated aquatic hyphomycete communities (Fig. 9) that – as hypothesised – might have triggered negative effects on the leaf palatability by favouring fungal species known to be rejected by *Gammarus* (cf. Arsuffi & Suberkropp, 1989; Bundschuh *et al.*, 2011). However, quinoxyfen differed from this pattern, as no apparent microorganism-induced food choice of *Gammarus* was observed (Figs. 9d and 11d). Additionally, effect sizes observed during the fungicide-adsorption experiments were in good agreement with those observed for the same test concentrations in the main experiment for all tested fungicides (Fig. 11). Since the fungicide-adsorption experiments excluded microorganism-induced food choices, we propose a repellent effect of leaf-adsorbed fungicides as the predominant driver for the observed feeding pattern (cf. Hahn & Schulz, 2007).

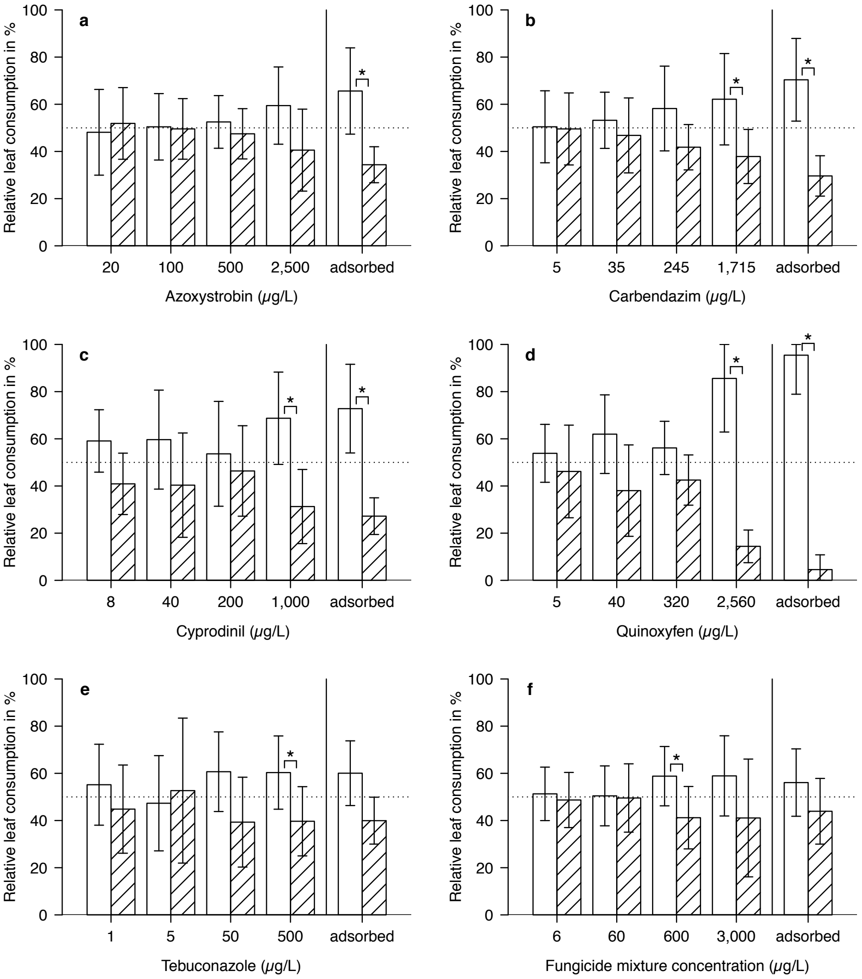

Figure 11. Mean or median relative consumption (with 95% CI) by *Gammarus fossarum* on leaf discs microbially colonised under control conditions (white bars) or exposed to different concentrations of (a) azoxystrobin, (b) carbendazim, (c) cyprodinil, (d) quinoxyfen, (e) tebuconazole, and (f) the fungicide mixture (hatched bars). "Adsorbed" refers to the additional experiments that addressed the potential repellent effect of leaf-adsorbed fungicides. Asterisks denote statistically significant differences to the respective controls. While 100% is the total leaf consumption in a food choice experiment, the horizontal line indicates the no-effect level (i.e. 50% consumption on both types of leaf discs; adapted after Zubrod *et al.*, 2015a).

Apart from effects provoked by the individual fungicides, their mixture also caused both structural and functional effects on microbial decomposers. The lowest mixture concentration (6 µg/L), which contained all five individually tested fungicides, already caused some 30% reductions in fungal species richness ($P = 0.040$) and bacterial abundance ($P = 0.044$; Fig. 9f). Despite this, however, a large fraction of the observed fungicide mixture effects (Figs. 10f and 11f) were lower than predicted by the model "independent action" (Table 2). A positive effect by quinoxyfen on aquatic hyphomycetes, indicated by the unexpected quinoxyfen-induced ≥ 30% increase in leaf-associated fungal biomass ($P \leq 0.028$; Fig. 10d), might be a plausible explanation for the discrepancy between predicted and observed effect sizes for microbial leaf decomposition (Table 2). This may be related to the ability of some fungal species to degrade and utilise xenobiotics as a source of energy (e.g. Krauss *et al.*, 2011; Hodkinson & Dalton, 1973). Additionally, leaf-associated fungi may have benefitted from the potentially reduced competitive pressure from bacteria caused by tebuconazole (Fig. 10e). Consequently, detoxification by aquatic hyphomycetes may to some extent have counteracted the adverse effects of the remaining fungicides in the mixture (cf. Solé *et al.*, 2012). Such investments would have lowered the energy available for biomass accrual and could therefore explain the absence of the expected increase in fungal biomass (Fig. 10f; Table 2).

Table 2. *Excerpt from the comparison of predicted (according to "independent action") and observed effect sizes (percentage reduction compared to control; Zubrod et al., 2015a). Agreement between prediction and observation was assumed, when the prediction was within the 95% CI of the observation (Rasmussen et al., 2012).*

Variable	Fungicide mixture concentration (µg/L)	Prediction	Observation (mean or median with 95% CI)	Mixture effect compared to prediction
Gammarus' leaf consumption	6	32.7	5.6 (-28.3 to 38.6)	Agreement
	60	48.2	-0.9 (-41.3 to 43.5)	Lower
	600	74.1	29.0 (5.7 to 53.7)	Lower
	3,000	87.8	34.5 (-22.0 to 92.8)	Agreement
Microbial leaf decomposition	6	-8.2	-3.9 (-8.3 to 0.5)	Agreement
	60	1.3	-0.1 (-5.1 to 4.8)	Agreement
	600	19.1	5.6 (1.1 to 10.2)	Lower
	3,000	32.2	12.1 (7.6 to 16.7)	Lower
Bacterial density	6	30.6	34.2 (2.9 to 56.8)	Agreement
	60	65.3	11.8 (-27.2 to 43.6)	Lower
	600	74.2	2.3 (-48.6 to 21.8)	Lower
	3,000	53.2	-4.6 (-225.9 to 25.7)	Lower
Fungal biomass	6	-156.9	9.6 (-34.8 to 27.1)	Lower
	60	-205.0	24.9 (0.8 to 35.0)	Reversed direction
	600	-39.9	42.4 (22.4 to 56.1)	Reversed direction
	3,000	62.1	54.3 (22.4 to 63.4)	Agreement

In conclusion, our findings show that fungicides induced both structural and functional effects in microbial communities. However, in contrast to our hypothesis, the alterations in aquatic hyphomycete communities likely did not affect the food choice of *Gammarus* (cf. conclusion of Paper II). Our absorbance experiments show that rather leaf-adsorbed fungicides had a repellent effect on *Gammarus*, resulting in lower feeding rates on fungicide-exposed leaf discs. Nevertheless, the alterations in aquatic hyphomycete communities and their biomasses may still lead to bottom-up effects on the physiological status of shredders, when they feed on fungicide-exposed leaf material over the long term while no alternative food is present. These effects may be corroborated by direct toxic effects of co-ingested fungicides and other leaf-adsorbed chemical stressors.

4.4 The relative importance of food-associated and waterborne fungicide exposure for leaf-shredders (Paper V)

As expected, the presence of epoxiconazole during microbial conditioning of leaves induced alterations in the leaf-associated microbial community. Fungal biomass was significantly reduced by approximately 25% compared to the control ($P = 0.033$). Also, bacterial abundance was slightly, albeit not significantly reduced by 15% ($P = 0.268$) upon epoxiconazole exposure. Besides effects on structural sum parameters of microbes, we found alterations in microbial metabolisation of C-substrates and shifts in microbial FA-profiles, which are indicators of changes in the microbial community composition. Metabolisation of all C-substrates was almost consistently higher in the fungicide treatment than in the control (Fig. 12). In other words, the number of utilised C-substrates was higher for epoxiconazole-exposed communities than for those in the control ($P = 0.011$). This pattern of higher C-metabolisation was likely caused by an epoxiconazole-induced community shift of microbes favouring generalists (Duarte *et al.*, 2008; Ratcliff *et al.*, 2006).

Also the observed epoxiconazole-induced difference in the microbial FA-profile indicates alterations in the aquatic hyphomycete community, given the reported differences in FA-profiles among individual aquatic hyphomycete species (Arce Funck *et al.*, 2015). Epoxiconazole-exposure did neither significantly affect the overall FA-profile of microbial communities ($P = 0.279$) nor the essential polyunsaturated FAs (PUFAs) and their derivatives ($P = 0.383$; including α-linolenic acid, arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid, and linolenic acid).

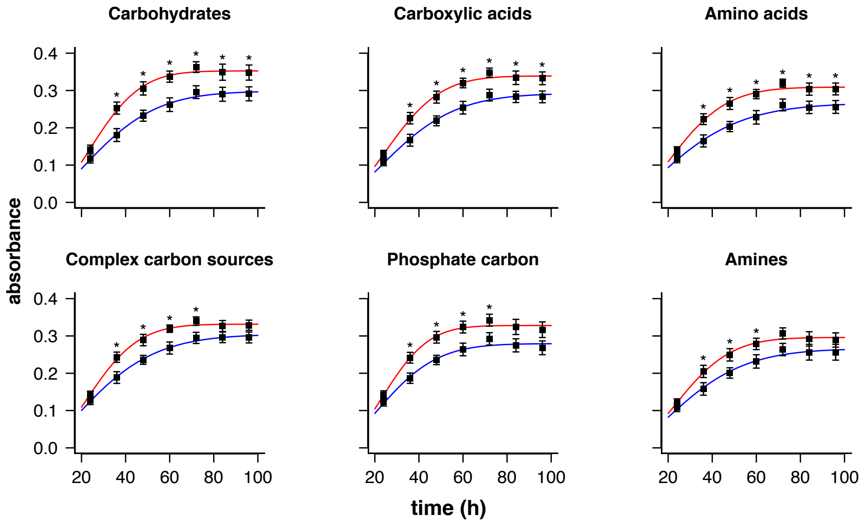

Figure 12. Time-series of absorbance measurements for the six carbon guilds metabolised by microbes on EcoPlates^{TM} over the incubation period of 96 h (n = 24). Bars represent standard errors around medians (black squares). Control and epoxiconazole treatment are displayed in blue and red, respectively, with asterisks indicating significant differences between them (adapted after Feckler *et al*., 2016).

However, fungicide-treated leaf material showed a 50% higher relative share of arachidonic acid (P = 0.025) and of eicosapentaenoic acid (P = 0.037) than that of the control. Accordingly, fungicide-induced effects on the leaf-associated microbial FA-profile were reported by Zubrod et al. (2017b), where the observed difference was primarily caused by altered contents of linolenic acid, α-linolenic acid, and eicosapentaenoic acid. These indicators of community shifts are underpinned by an up to eight-fold higher toxicity of epoxiconazole to aquatic fungi compared to tebuconazole (Dijksterhuis *et al.*, 2011) and the observed effects on aquatic hyphomycete composition at slightly higher tebuconazole concentrations (Paper IV).

Asellus' leaf consumption and excretion within each treatment were jointly interpreted as a proxy for the assimilation of ingested energy (difference between consumption and excretion), although this approach ignored respiration of test organisms (cf. Graça *et al.*, 1993a). When feeding on fungicide-treated leaf material (i.e. affected *via* the indirect pathway), *Asellus* showed a slightly increased leaf consumption (~5%; P = 0.591) and reduced excretion (~20%; P = 0.118). Although these individual results were not significant, they may indicate a biologically relevant increase of 25% in energy assimilation compared to the control. *Asellus* exposed directly through the water phase reduced their average leaf consumption by ~5% (P = 0.044) and their excretion by ~10% (P = 0.357) suggesting a slightly increased assimilation of the ingested energy (~5%). An increased assimilation may point to an enhanced energy utilisation, particularly when feeding on fungicide-treated leaf material (~25% higher assimilation), implying *Asellus* builds up higher energy reserves (=lipids) and/or grows faster. However, *Asellus'* growth during the experiment was some 30% lower when feeding on fungicide-treated leaf material (P = 0.262) and some 40% lower when directly exposed through the water phase (P = 0.116; Fig. 13a) than in the control. Although these measurements showed high variability (Fig. 13a) and results were not significant, a 30-40% reduction in growth can still be seen as biologically relevant. *Asellus'* overall lipid content was also marginally lower when feeding on fungicide-treated leaf material (~10%; P = 0.447) and directly exposed through the water phase (~20%; P = 0.030; Fig. 13b) than in the control. The realistic worst-case (combined) scenario in our experiment, during which *Asellus* fed on fungicide-treated leaf material while exposed to the fungicide through the water, resulted in an additive action between the two pathways. Energy assimilation was unaffected because of some 15% reductions in both the consumption and excretion compared to the control. However, the growth and lipid content of *Asellus* were 45% and 30%, respectively, lower in the combined scenario than in the control (Fig. 13a&b). This suggests an increased energy expenditure under toxic stress due to detoxification (Maltby, 1999) when exposed directly or due to co-ingestion of leaf-adsorbed epoxiconazole.

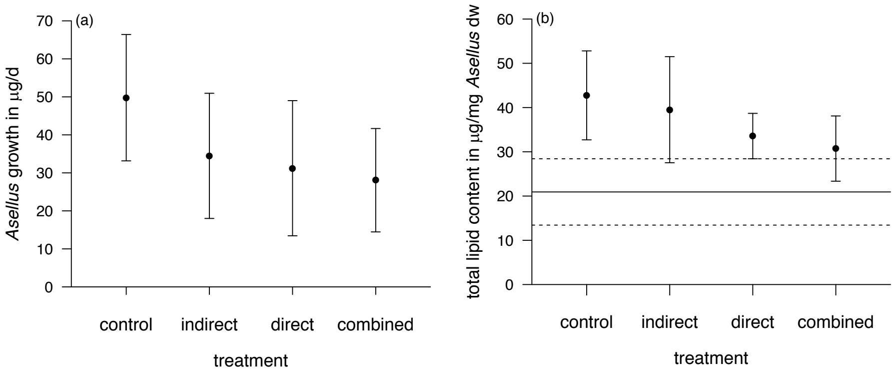

Figure 13. Mean (with 95% CI) growth (a) and lipid content (b) of *A. aquaticus* subjected for 28 days to four treatments: the control (epoxiconazole-free), the indirect (diet-related effects), the direct (waterborne toxicity), and the combined exposure scenario. The solid, horizontal line in Fig. 13b indicates the mean initial lipid content of *A. aquaticus* (with 95% CI; indicated by dashed, horizontal lines) at the set-up of the experiment (Feckler *et al.*, 2016).

Finally, the ~25% reduced fungal biomass associated with leaf material conditioned in the presence of fungicides likely provided a lower food quality for *Asellus*. This suggestion is based on the nutritional value of microbial cells, which is 4 to 10 times higher than that of unconditioned leaf material (Bärlocher & Kendrick, 1975), and the high contribution of fungi to the microbial biomass on decaying leaves (Gessner *et al.*, 2007).

Asellus' FA-profiles differed between the control and the indirect (P = 0.039) and the direct pathway (P = 0.014) as well as the combined scenario (P = 0.003), which could provide a mechanistic interpretation of the effects on *Asellus'* physiology. On one hand, individual saturated FAs showed an up to ~60% reduction or almost depletion likely suggesting their usage for catabolic processes (as reviewed in Tocher, 2003). On the other hand, oxidative stress-related peroxidation that results in degradation of unsaturated FAs (as reviewed in Halliwell & Gutteridge, 1984) could explain the observed negative trend for some monounsaturated FAs and PUFAs (including arachidonic acid and eicosapentaenoic acid). Given the fundamental role especially of these PUFAs for invertebrate growth and maintenance (Parrish, 2009), such effects on the FA-profile may explain the lower lipid content and growth of *Asellus* affected by epoxiconazole.

In conclusion, waterborne effects on *Asellus* were generally stronger than the diet-related counterparts. Nevertheless, both pathways are of biological relevance from a food web perspective. This is because of the sorption of many pesticides on organic matter (such as leaf material) in surface waters (sensu Weber, 1972) and consequently their dietary uptake during consumption and effects on the microbial community (cf. conclusion of Paper IV). Ignoring the less pronounced indirect pathway may thus result in an underestimation of effects on the function and physiology of leaf-shredders.

5 Conclusions and future perspectives

This thesis is based on a series of experimental studies of aquatic microbial decomposers and primary consumers (i.e. leaf-shredding detritivores) mediating leaf litter decomposition in detritus-based food webs. This setting allowed me to quantify fungicide effects on ecosystem processes performed by these organism groups as well as their interaction. Overall, our studies show that fungicides at field-relevant concentrations can provoke detrimental effects in the structure and function of both decomposers and detritivores (Papers II-V). From these results, we conclude that effect sizes may increase in the future due to predicted higher fungicide concentrations resulting from agricultural intensification. This indicates consequences for the energy flow in detritus-based food webs due to their strong bottom-up regulation. The observed fungicide-induced reduction in leaf decomposition by previously non-exposed decomposer communities and detritivores (Papers II-V) limits the availability of leaf-bound energy for the remaining food web or slow down energy metabolism. Although our results suggest that fungicide effects on decomposers were buffered through the development of a pollution-induced community tolerance (Papers II & III), the associated community shifts and biodiversity loss might limit the communities' plasticity to respond to additional stresses. Moreover, detritivores' faeces constitute a high-quality food source in aquatic ecosystems (Fig. 1). The observed reduction in faeces production by detritivores when feeding on leaf material of lower quality or being exposed to fungicides through the water (Paper V) therefore suggests effects on the food availability for collecting invertebrates. Finally, the impairments in the overall lipid content and growth of *Asellus* (Paper V) could ultimately have consequences for food webs, as detritivores such as *Asellus* represent a key prey for various aquatic and terrestrial predators.

The fungicide use in existing agricultural areas should therefore be reduced. This could, for example, be accomplished by promoting alternative cultivation methods such as "precision farming" that accounts for the variability and uncertainties within agricultural systems and allows for a more sustainable agricultural production (Gebbers & Adamchuk, 2010). In addition, edge-of-field buffer strips (Reichenberger *et al.*, 2007), vegetative drainage ditches (Bennett *et al.*, 2005) and constructed wetlands (Schulz & Peall, 2001) could help to minimise pesticide leakage to aquatic ecosystems. Moreover, agricultural expansion into currently unpolluted areas should be restrained by, for example, producing food more efficiently, reducing food waste, and optimizing the resiliency of agriculture and food production (Laurance *et al.*, 2014). These innovative approaches would ultimately help to safeguard the integrity of ecological processes delivered by microbial decomposers and detritivores.

This thesis work identified several knowledge gaps that are relevant for future research. As pointed out in Paper IV, several fungal and fungal-like groups of organisms such as Chytridiomycota, Oomycota, and Zygomycota are suggested to contribute to leaf decomposition (cf. Manerkar *et al.*, 2008; Nikolcheva & Bärlocher, 2004). These organism groups are typically not addressed in leaf litter decomposition research, either because they are hard to identify using microscopic techniques or do not produce ergosterol. Incorporating these groups of organisms into experimental schemes, as applied during the present thesis, should foster a better understanding of stressor-induced effects in decomposer communities. Identification of these groups can be achieved by integrating molecular biological methods such as next-generation sequencing (Lindahl *et al.*, 2013) in combination with species-specific quantitative real-time polymerase chain reaction (qPCR; Feckler *et al.*, 2017). This approach provides a better resolution of the community composition and performance on individual decomposer species than traditional methods (spore counts and ergosterol) do. The use of qPCR will especially help to develop a better understanding of individual fungal species' contribution to leaf decomposition under stress as the biomass for each species can be quantified accurately. This knowledge will also better elucidate the linkages between fungal diversity and ecological functions under chemical stress.

Furthermore, a combined approach of experimental procedures such as those applied during the present thesis as well as qPCR might result in a better mechanistic understanding of trophic relationships between shredders and leaf-associated fungal species (Arsuffi & Suberkropp, 1989). Previous studies on the effect of chemical stressors on the food choice of leaf-shredders (Zubrod *et al.*, 2015a; Bundschuh *et al.*, 2011; Bundschuh *et al.*, 2009) were limited in their explanatory power, because interpretations were based on spore counts, which does not necessarily correlate with the biomass of the respective aquatic hyphomycete species (Bermingham *et al.*, 1997). Applying qPCR could provide deeper insights into leaf-shredders' food preferences by estimates of single-species biomasses linked to prior knowledge of preferred fungal species.

Other important knowledge gaps are the effects caused by chemical stressors' adsorption to leaf litter, their co-ingestion during feeding and subsequent effects on detritivores, given the observed effects during this thesis (but see also Zubrod *et al.*, 2015b; Dimitrov *et al.*, 2014; Bundschuh *et al.*, 2013). Toxicokinetic-toxicodynamic modelling (e.g. Ashauer *et al.*, 2011) that encompasses the uptake, distribution and effects of chemical stressors could help to better understand implications on individual organisms and extrapolate these to the population level.

Also, the interaction of press and pulse disturbances in multiple-stressor scenarios is, to date, not well studied (but see e.g. Salo *et al.*, in press; Zubrod *et al.*, 2017a). On one hand, stream-inhabiting organisms are subjected to constant-press disturbances such as micropollutants in wastewater treatment plant effluents (Gago-Ferrero *et al.*, 2017). On the other hand, pulse disturbances such as short-termed pesticide run-off from agricultural fields (Schulz, 2014) and heat waves as a consequence of climate warming (Kirtman *et al.*, 2013) can put additional stress on organisms already exposed to micropollutants. Therefore, assessing the plasticity and resilience of decomposer communities and detritivore populations in multiple-stress scenarios should be an interesting topic for future research. However, predicting the response of organisms in multiple-stress scenarios is problematic, as the individual stressors may not only show an additive interplay, but can interact with each other and result in synergistic or antagonistic effects (Folt *et al.*, 1999). Moreover, some stress effects possibly manifest after a lag time due to delayed effects on individuals (Stoks & Córdoba-Aguilar, 2012) or trans-generational effects (e.g. Li *et al.*, 2016). It therefore seems crucial to understand the resilience of decomposer communities and detritivore populations under multiple-stress scenarios to ultimately safeguard the functional integrity of detrital food webs.

A final research perspective is to gain better insight in fungicide effects in the field (e.g. Fernández *et al.*, 2015), by simulating more environmentally realistic scenarios under laboratory conditions. This could include the testing of higher levels of biological organisation such as detritivore communities consisting of species exhibiting differing sensitivities to chemical stressors and a gradient of functional traits. The latter seems especially important, as detritivores' functional composition and diversity, in terms of functional dispersion and identity, strongly affects leaf litter decomposition through functional complementarity and trait-dependent effects (Frainer & McKie, 2015). Such an approach may ultimately help to unravel the discrepancies in fungicide effects on detritivores observed in laboratory studies (as conducted in this thesis) and those observed in the field (e.g. Fernández *et al.*, 2015).

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Popular science summary

One of the most common questions I was asked by my family and friends over the last few years was ”What are you studying exactly?”. I tried to make it easy and answered “Environmental Sciences”, which most of the times resulted in the comment “Ah, wonderful, something with environment. This is important these days” – and that’s where the conversation ended. To finally put it into perspective, this is what I have actually been doing during the past four years: I studied how fungi, bacteria, and little invertebrates that live in streams are affected by fungicides we use in agriculture.

But let us start from the beginning… Imagine you are standing next to a stream surrounded by trees that cast a shadow on the water. The shade from the trees hinders the growth of algae, since these organisms need light to convert into energy during photosynthesis and fuel their activity – a process you may recall from biology classes in school. Consequently, organisms living in these streams cannot use algae as a high-quality food source; leaves falling from the trees are instead known as the main source of energy. Before the majority of stream-inhabiting organisms can use the energy stored in leaves, they have to be processed by microbes (bacteria and fungi) and small invertebrates, so-called “leaf-shredders”. The latter organisms fragment the leaf material while feeding on it – like the Cookie Monster from the Sesame Street does when eating cookies and spreading crumbs all over the place. However, chemicals that we apply to increase our crop yields, for example fungicides that kill fungal pests, can enter these streams and cause harm to microbes and leaf-shredders. Both groups of organisms can be affected when they are directly in contact with fungicides through the water. Besides, fungicides can stick to the leaf material and can be ingested by leaf-shredders during feeding. Finally, fungicide effects on the growth and assemblage of fungi on the leaf material can indirectly affect leaf-shredders, since they prefer to eat leaves overgrown by fungi – just as we prefer bread with jam over plain bread – and prefer to eat some fungi more than others. Coming back to the beginning, you now understand why I looked into the question of how fungicides affect microbes, leaf-shredders, and the interplay between them. But what did I find out?

My studies showed that fungi did not grow as well and changed their assemblage when in contact with fungicides, which eventually affected their processing of leaf material. When I studied microbes that had not been in contact with chemicals before, fungicides decreased the processing of leaf material. On the other hand, I surprisingly found that a previous exposure to chemicals in streams increased microbes' processing of leaf material when they were exposed to fungicides in the laboratory. You likely know a similar adjustment of your own performance: when you train at the gym for the first time, your muscles are sore for the next few days and you move slower than usual. However, with time and more exercise, your body adapts to the training and you can lift more weight. An increased processing of leaf material after adaptation of microbes to chemicals was not only observed in Sweden, but was also found in Denmark and Germany, suggesting this pattern as a general response of microbes. Moreover, fungicides influenced the food quality for leaf-shredders: On one hand, fungicides sticking to the leaves reduced their taste for leaf-shredders. On the other hand, the reduction of fungal growth on the leaf material reduced the ”jam on the bread” and lowered the food quality. When feeding on these untasty and low-quality leaves, leaf-shredders grew less and stored less fat. This happened also when leaf-shredder were in contact with fungicides through the water, but in this situation their growth and fat storage was even more reduced. Although the effects on leaf-shredders were stronger when they were in direct contact with the fungicides through the water, we can not ignore the food-quality related effects. This is because many fungicides stick to leaves and accumulate when they enter the streams. This affects the food quality for leaf-shredders and they are taken up involuntarily during feeding. Additionally, leaf-shredders can feed on leaves of lower quality and be in contact with fungicides through the water at the same time. In this case, the effects caused by the lower food quality and the fungicide effects over the water were combined and reduced the growth and fat storage even more.

So, this is what I have been doing during the past four years in Sweden. Maybe you will think of the Cookie Monster, bread with jam or your gym when you pass by a small stream and are reminded of my research.

Populärvetenskaplig sammanfattning

En av de vanligaste frågorna jag fått från min familj och vänner under de senaste åren var ”Exakt vad är det du studerar?”Jag försökte göra det enkelt och svarade ”Miljövetenskap”, vilket oftast resulterade i svaret ”Åh, underbart, något med miljö. Det är viktigt dessa dagar” – och där tog sedan samtalet slut. För att nu till sist sätta det i sitt rätta sammanhang – detta är vad jag faktiskt har gjort under de senaste fyra åren: Jag studerade hur svampar, bakterier och små ryggradslösa djur som lever i vattendrag påverkas av svampbekämpningsmedel (även kallade svampmedel) som vi använder inom jordbruket.

Men, låt oss börja från början… Tänk dig att du står alldeles bredvid ett vattendrag som omges av träd som kastar en skugga över vattnet. Skuggan från träden hindrar algerna från att växa, eftersom grunden för deras utveckling är att de behöver solljus som kan omvandlas till energi genom fotosyntesen – en process du kanske kommer ihåg från biologilektionerna i skolan. Organismer som lever i dessa vattendrag kan därför inte använda alger som en fullvärdig energikälla; löv som faller från träden blir istället deras främsta energikälla. Innan de flesta vattenlevande organismerna kan livnära sig på löven måste de dock först bearbetas av mikrober (bakterier och svampar) och små ryggradslösa djur, så kallade ”lövfragmenterare”, som finfördelar löven samtidigt som de livnär sig på dem – precis som Kakmonstret i ”Sesame Street” gör när han äter kakor och strör smulor överallt. Kemikalier som vi använder för att öka våra skördar, till exempel svampmedel som tar död på skadesvampar, kan hamna i våra vattendrag och orsaka skador på mikrober och lövfragmenterare. Båda dessa organismgrupper kan påverkas när de kommer i direkt kontakt med svampmedel i vatten. Dessutom kan svampmedel fastna på lövmaterialet och tas upp av lövdelarna som de mumsar på. Svampmedel kan påverka både tillväxt och svampsammansättning på löven vilket indirekt kan påverka lövfragmenterare, eftersom de föredrar att äta löv som är övervuxna av svampar – på samma sätt som vi föredrar bröd med sylt i stället för bara bröd.

Det är också så att organismerna föredrar att äta vissa svampar framför andra, precis som vi. För att återvända till inledningen – nu förstår du varför jag intresserat mig för frågan om hur svampmedel påverkar mikrober, lövfragmenterare och samspelet mellan dem. Men, vad har jag kom fram till?

Mina studier visade att svamparna inte växte lika bra och att sammansättning av svampar förändrades när de kom i kontakt med svampmedel. Detta ledde så småningom till att nedbrytningen av löven påverkades. Mikrober som inte hade varit i kontakt med kemikalier tidigare minskade sin bearbetning av lövmaterial när de utsattes för svampmedel. Å andra sidan fann jag till min överraskning att om mikroberna tidigare hade exponerats för kemikalier i vattendrag så ökade bearbetningen av lövmaterialet när de därefter utsattes för svampmedel- den här gången i laboratoriet. Du känner säkert igen en liknande tillvänjning av din egen prestation. När du tränar i gymmet för första gången är dina muskler ömma de närmaste dagarna och du går långsammare än vanligt. Men med tiden och med mer träning anpassar sig din kropp till träningen och du kan lyfta tyngre vikter. En ökad bearbetning av lövmaterial efter att mikroberna anpassat sig till kemikalier observerades inte bara i Sverige, utan också i Danmark och Tyskland. Detta beteende hos mikroberna följer alltså ett generellt mönster. Dessutom visade det sig att svampmedlen påverkat kvalitén på lövfragmenterarnas mat. Å ena sidan så minskade svampmedlen som fastnade på löven deras smak för lövfragmenterare. Å andra sidan innebar den minskade svamptillväxten på bladmaterialet en minskad "sylt på brödet" effekt och därmed sänkt matkvalitet. När lövfragmenterare utfodrades med dessa löv som både smakade dåligt och var av låg kvalitet så växte de mindre och lagrade mindre fett. Samma sak hände också när lövfragmenterare kom i kontakt med svampmedel direkt genom vattnet, men då blev deras tillväxt och fettlagring ännu lägre. Trots att effekterna på lövfragmenterare var kraftigare när de var i direkt kontakt med svampmedlet i vattnet, får vi inte bortse från de födorelaterade effekterna. Detta eftersom svampmedel gärna fäster till löven och ackumulerar där. Detta leder till väsentligt försämrad födokvalitet för lövfragmenterarna och en ofrivillig konsumtion av svampmedel. Dessutom sker de negativa processerna samtidigt; konsumtion av löv av lägre kvalitet och direkt kontakt med svampmedel växelverkar på ett negativt sätt, vilket gör att effekten på tillväxt och fettinlagring blir ännu större.

Så detta är vad jag har gjort under de senaste fyra åren i Sverige. Kanske kommer du att tänka på Kakmonstret, bröd med sylt eller ditt gym nästa gång du passerar ett litet vattendrag eller så blir du påmind om min forskning.

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"The best education I have ever received was through travel"
(Lisa Ling)

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