The relevance of the biodiversity to function relationship in heterotrophic aquatic systems under stress

by

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Declaration

I hereby declare that I independently conducted the work presented in this thesis entitled “The relevance of the biodiversity to function relationship in heterotrophic aquatic systems under stress”. All used assistances are mentioned and involved contributors either are co-authors of or are acknowledged in the respective publication. Artificial intelligence tools have not been used in any part of this thesis. Moreover, this thesis has never been submitted elsewhere for an examination, as a thesis or for evaluation in a similar context to any department of this university or any scientific institution. I am aware that a violation of the aforementioned conditions can have legal consequences.

Dübendorf, 30/08/2024

Place, date Signature

The results presented in this cumulative thesis are documented one manuscript that is currently under revision (**Appendix I**) and in two peer-reviewed publications (**Appendix II and III**). Since many researchers contributed to the work I present in this thesis (see **Appendix IV**), I will use the plural form when presenting and discussing these results.

Appendix I

Gonçalves, S., Post, R., Konschak, M., Zubrod, J., Feckler, A., & Bundschuh, M. (2023). Leaf Species-Dependent Fungicide Effects on the Function and Abundance of Associated Microbial Communities. Bulletin of Environmental Contamination and Toxicology, 110(5), 1–7. https://doi.org/10.1007/s00128-023-03728-2

Appendix II

Gonçalves, S., Feckler, A., Pollitt, A., Baschien, C., Michael, J., Schreiner, V. C., Zubrod, J. P., Bundschuh, M. (2024). Elevated Fungicide and Nutrient Concentrations Change Structure but not Function of Aquatic Microbial Communities. Environmental Toxicology and Chemistry, 43(6), 1300–1311. https://doi.org/10.1002/etc.5863

Appendix III

Gonçalves, S., Pollitt, A., Pietz, S., Feckler, A., & Bundschuh, M. (2024). Microbial community history and leaf species shape bottom-up effects in a freshwater shredding amphipod. Science of the Total Environment, 912, 168926. https://doi.org/10.1016/j.scitotenv.2023.168926

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This is probably the shortest thank you I can write, but I do have to thank the many people who directly and indirectly contributed to this work and my wellbeing during this chapter. During the PhD we get absorbed in our research, “Am I do it right? Is it good enough? Is it relevant?” we always want more and forget that life is running on the sidelines. Life goes on, people come and go, you come and go, PhD finishes, and the only (important) things that stay are the skills developed and the extraordinary and inspiring people you meet. I am grateful to all of you for the unique and unforgettable memories.

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Contents

ABSTRACT

Expansion of agricultural land-use and the associated application of agrochemicals can have deleterious effects on local freshwater microbial communities, with consequences for the entire ecosystem. Leaf litter decomposition is a key process in stream ecosystems being partially driven by microbial communities (particularly fungi and bacteria). Leaf-associated microbial communities are responsible for making the nutrients and energy bound in leaves available for higher trophic levels of heterotrophic food webs. Moreover, these microbial communities increase the nutritional quality of leaf litter for shredders, as they produce proteins and lipids while degrading the indigestible components of leaf litter. When exposed to anthropogenic pressures, such as the increased exposure to nutrients and fungicides associated with agricultural land-use, the structure and function of these microbial communities can be affected. In addition, the leaf species on which these microbial communities grow may act as a supplementary filter for the community structure and response to stressors. These factors and their interaction may jointly modify leaves' nutritional quality for higher trophic level, potentially affecting activities such as shredders' feeding and development. Despite the importance of leaf litter decomposition, little is known about the underlying mechanisms or processes driving the changes in function and structure (mainly in the aquatic hyphomycetes [AH] community) of leaf-associated microbial communities. Moreover, fungicide effects on leaf litter decomposition were investigated almost exclusively with black alder leaves due to their favourable traits to consumers (i.e., low recalcitrance and high nutrient content). Simultaneously, little is known about fungicide effects on microbial colonisation and decomposition of other leaf species, with less favourable traits or potential unknown consequences for the wider food web. The aims of this thesis are therefore to assess individually: - the effects of fungicide exposure on leaf-associated microbial communities colonising different leaf species; - the effects of combined fungicide and nutrient exposure on microbial communities with different exposure history; - the potential effects on shredders' development resulting from feeding on different leaf species colonised by communities with different exposure history. These aims were assessed through a set of complex laboratory bioassays taking into account the environmental relevance of the tested stressors and communities.

Overall, we show that microbial communities colonising leaves with less favourable traits (i.e., higher recalcitrance and lower nutrient levels such as European

beech) potentially may suffer increased fungicide effects, affecting their function (i.e., leaf litter decomposition). While leaf species with more favourable traits such as black alder, enabled leaf-associated microorganisms to acquire leaf-bound energy and more easily resist potential effects induced by fungicide exposure. Moreover, our results also point towards the need to expand our mechanistic understanding on how different leaf species interact with the effects of chemical stressors on the function and structure of microbial communities. The latter is not only important due to the expected changes of leaf species input into streams but also because those can potentially translate into different food quality for shredder organisms. Secondly, leaf litter decomposition did not differ between fungicide treatments or exposure histories. While increasing levels of nutrients tended to buffer for the non-significant fungicide-induced effects on leaf decomposition. However, fungal community composition substantially changed at environmentally relevant fungicide concentrations. For example, in most communities tolerant AH species of the genus *Tetracladium*, known by its superior leaf decomposition efficiency, dominated at high fungicide exposure independent of exposure history. Since the changes in the fungal community composition seem decoupled from its function, our results are therefore supporting the principle of species dominance. This principle elaborates that highly efficient decomposers are responsible for maintaining leaf litter decomposition despite changes in the community structure. However, changes in the community structure can potentially affect other functions provided by fungi, such as increasing the nutritional quality of leaves for shredders. Finally, we also show that leaf species identity has a more substantial impact on gammarids' development relative to the exposure history of the microbial community colonising the leaves. Moreover, the sex-specific feeding responses of gammarids raise questions on earlier procedures, demanding further research.

1. INTRODUCTION

In forest-dominated catchments, stream ecosystems are maintained by the decomposition of allochthonous organic carbon, mainly subsidized in the form of terrestrial leaf litter (Fisher & Likens, 1973; Minshall, 1967; Nelson & Scott, 1962). In such environments, the leaf litter is colonised by aquatic microorganisms, such as aquatic hyphomycetes (AH; a polyphyletic group of asexual fungi; Baschien et al., 2006) and bacteria (Gessner et al., 1999). In this context, microbial communities' efficiency to colonise and consequently decompose leaf litter is assumed to be mainly a function of their fungal species-specific traits (Baudy et al., 2021), as well as the chemical composition of leaf species used as substrate (Hladyz et al., 2011; Melillo et al., 1982; Schindler, M. H., 2009). In fact, nutrients and structural components of leaves (i.e., recalcitrant components such as lignin) can influence microbial colonisation dynamics (Gessner & Chauvet, 1994; Melillo et al., 1982; Webster & Benfield, 1986). Once the leaf litter colonisation is successful, these microorganisms produce exoenzymes responsible for breaking down mono-, di- and polysaccharides into more usable and accessible compounds for the higher food web (Evans & Hedger, 2001; Hieber & Gessner, 2002). Moreover, this conditioning process by bacteria and fungi increases the leaves' nutritional quality and palatability for leaf-shredding invertebrates. The microbial conditioning indirectly promotes leaf litter decomposition through the stimulation of shredders' feeding activity (Bärlocher & Kendrick, 1975; Cummins, 1974). This stimulation of feeding ultimately results in the production of fine particulate organic matter, an essential resource for collectors and deposit-feeding organisms (Bundschuh & McKie, 2016). Thus, driven by the crucial role in stream food webs, changes in leaf-associated microbial communities can have far-reaching ecological consequences (Gessner et al., 2010).

The type of substrate used for colonisation (i.e., leaf species identity) may act as a filter for leaf-associated microbial communities due to leaf species' unique recalcitrance and nutrient levels. Most of the studies assessing impacts of chemicals, such as fungicides, on this type of communities used black alder (*Alnus glutinosa* (L.) Gaertn.) as a model leaf species. Black alder is considered representative of temperate riparian ecosystems (Bjelke et al., 2016); however, other leaf litter species are also ecologically highly relevant as they are present in the riparian ecosystem (Gessner et al., 2010). Black alders' richness in nutrients and relatively low share of recalcitrant substances (Gulis, 2001; Melillo et al., 1982) supports microbial growth and activity

through an easy access to nutrients and thus being the first to be colonised and decomposed by the microbial communities (Artigas et al., 2004; Graça & Canhoto, 2006). On the other hand, other leaf species with less favourable traits (i.e., lower nutrient content and higher content in recalcitrant substances) are colonised and decomposed slower, enabling the constant input of nutrients all year long (Gessner et al., 2010). As a result, these different leaf traits may question the transferability of results obtained with black alder-associated microbial communities exposed to stressors to other leaf litter species with deviating traits.

At the same time, the structure and function (i.e., leaf litter decomposition) of leaf-associated microbial communities is shaped by the surrounding environment, for example by the type of catchments' land-use, which can influence chemical input of anthropogenic origin (Canhoto et al., 2016). A repeated or continuous exposure to anthropogenic chemicals, for instance due to agricultural land-use, is characterised by the released of pesticides such as fungicides (Tilman et al., 2001) and nutrients. This type of exposure can trigger changes in leaf-associated microbial communities' function and structure (Feckler et al., 2018; Fernández et al., 2015). While fungicides mainly reduced leaf litter decomposition (Fernández et al., 2015), nutrients, on the other hand, are generally associated with a higher microbial activity (Ferreira et al., 2015). Additionally, previous studies have shown that communities' exposure history impacts their functional response to stressors (i.e., fungicides and nutrients, Feckler et al., 2018). In this context, Feckler et al. (2018) have shown that communities with exposure history, such as impacted by agriculture, compared to communities without exposure history, from near-natural streams, displayed higher functional (leaf litter decomposition) tolerance towards fungicides. The latter findings suggest that a history of exposure to nutrients and fungicides may also act as a filter selecting for tolerant (and partly more efficient) species, in this case of AH species, as they are considered major drivers for leaf litter decomposition (Gessner et al., 2007). Although Feckler et al. (2018) findings have been straightforward, its applicability required an expansion of true microbial communities' replicates (independent natural communities) with and without an "exposure history".

In addition, leaf litter palatability and its nutritional quality for shredders has been shown to be modified under constant exposure to fungicides (Fernández et al., 2015; Konschak et al., 2020; Zubrod et al., 2015). However, it remains unclear if changes in microbial communities and nutritional quality of leaves prevail after long-term field

exposure to fungicide peaks (i.e., even when communities and leaves are no longer actively exposed to fungicides). Once more, most of the studies assessing the direct effects of fungicides used black alder as substrate for microbial colonisation and shredders' feeding. It may therefore be questioned whether the effects observed using black alder are transferable to leaf species with differing traits (lower nutrient contents and/or higher degree of recalcitrance).

2. RESEARCH QUESTIONS

Despite the growing number of studies exploring the effects of stressors on leaf litter-associated microbial communities, our mechanistic understanding of how these communities respond to different stressors, how stressors and other factors can influence microbial function and structure as well as potential induced changes on trophic relationships is still limited. The main goal of this thesis was to create and explore data on the direct and indirect effects of multi-stressors (fungicides and nutrients) and factors as land-use (i.e., exposure history) and different substrate (i.e., leaf species) on aquatic microbial communities associated with leaf litter and their direct consumers. Previous studies mostly used only one type of leaf species as a substrate for microbial colonisation to assess effects of different stressors. However, this is hardly the case found in natural environments, where microbes colonise whatever mixture of leaves it is available. Moreover, different studies have shown the effects of fungicides and nutrients, alone or in combination on leaf-associated microbial communities; however, those studies mostly focus on one type of community, having very few environmental field replicates, or used single species of fungi. Additionally, previous studies focused on the direct effects of fungicides on primary consumers, while the indirect effects (e.g., through dietary exposure) and underlying mechanisms remain unclear.

In this thesis, we tried to address these knowledge gaps, bringing to light the following research questions in the respective papers:

I. Effects of fungicides on leaf-associated microbial communities colonising different leaf species: in presence of fungicides, are the microbial communities colonising different types of leaf litter (different quality) equally suffering the same structural and functional changes? (**Appendix I**).

II. Effects of combined exposure to fungicides and nutrients on leaf-associated microbial communities with differing exposure history: Is a different exposure history influencing structural and consequently functional responses to stressors of leaf-associated microbial communities? (**Appendix II**).

III. Microbial community exposure history and leaf species effects on *Gammarus fossarum:* Are primary consumers such as shredders affected by different food sources derived from leaf associated microbial communities colonising different leaf species (**Appendix I**)? Is community exposure history (**Appendix II**) acting as an additional factor with potential consequences for wider trophic levels? (**Appendix III**).

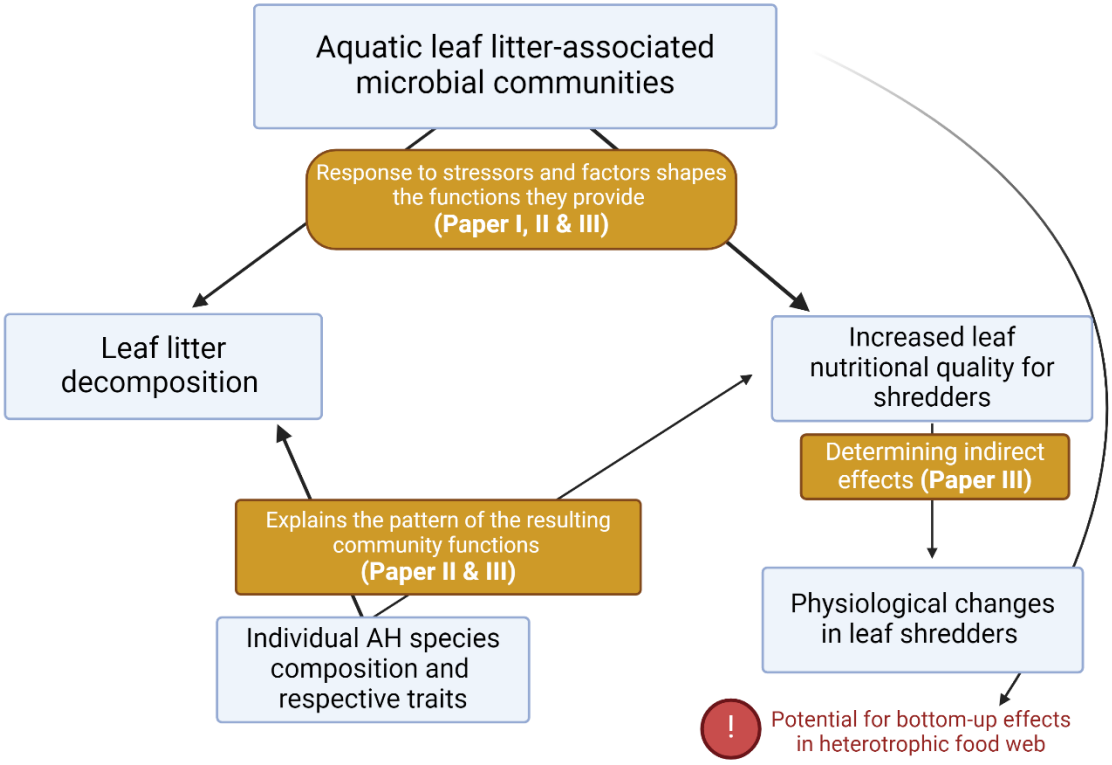

Figure 1. Conceptual overview of the research questions (**Papers I, II, III**) in this thesis.

3. MATERIAL AND METHODS

3.1 General experimental designs

Paper I - Effects of fungicides on leaf-associated microbial communities colonising different leaf species

In this study, leaf species with distinct traits were used: black alder, with relatively higher content in nutrients and lower in recalcitrant substances, compared to Norway maple (*Acer platanoides* L.) and European beech (*Fagus sylvatica* L.; Abelho, 2001; Gessner & Chauvet, 1994; **Appendix I**) respectively. Leaf material was collected in the same region, as in Paper II, and stored at -20 ºC until use. The leaf-associated microbial community was generated using alder leaves in mesh bags deployed in a pristine stream for 14 days (Fig. 2 – Step 1). In the laboratory, the same leaves were acclimatised and homogenised to prepare a microbial inoculum for the exposure assay (Fig. 2 – Step 2; see details in material and methods **Appendix I**).

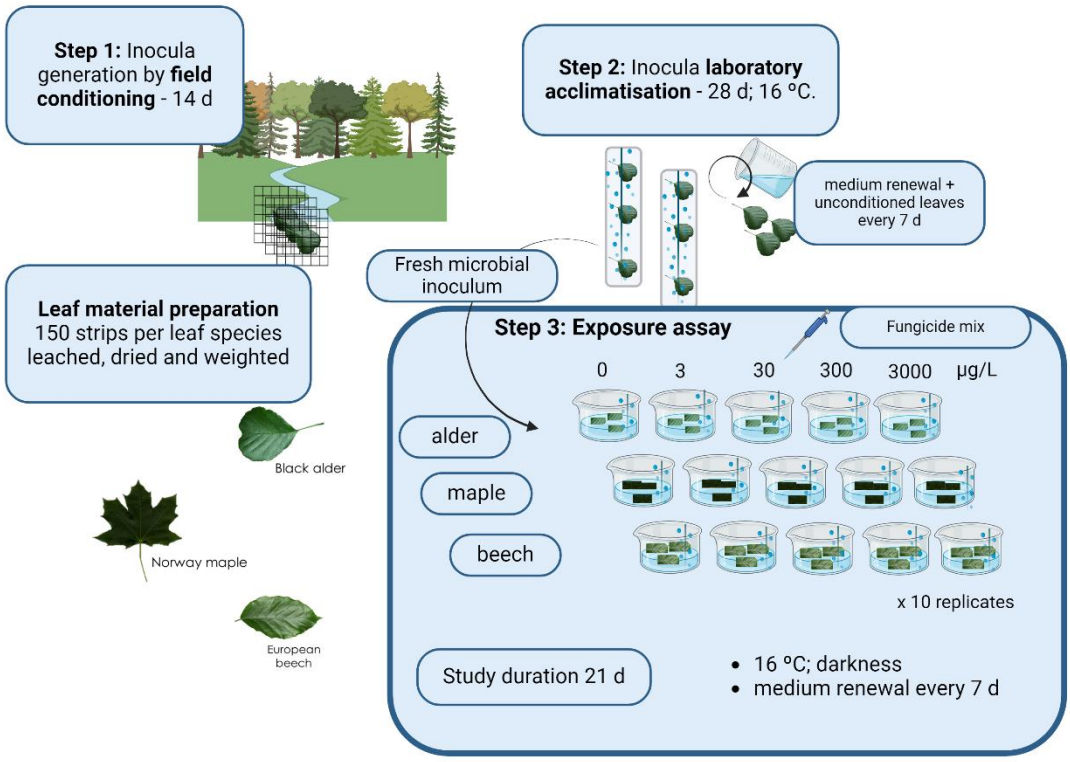

Figure 2. Overview of the study design. Step 1: Generation of inocula from a near-natural stream, Rodenbach, Germany (49°33´N, 8°´2´O) for 14 d; Step 2: Inocula acclimatisation to laboratory, leaves are cleaned and conditioned in channels for 28 d with medium renewal and addition of unconditioned leaves every 7 d; Step 3: Exposure assay- the generated inocula was used to condition the pre-experiment prepared leaf strips from 3 different leaf species: black alder; Norway maple and European beech. In 1 L beakers, leaf strips were exposed to increasing concentrations fungicides, over 21 d with medium and fungicide renewal every 7 d. Created with BioRender.com

Five fungicides, covering a wide range of modes of action, were used and the chosen concentrations followed earlier studies (e.g., Zubrod et al., 2015, Table 1): 0 (fungicide-free control), 3, 30, 300 and 3000 µg/L. For each leaf species, 150 strips were cut out from unconditioned leaves, dried and pre-weighed, leading to a total of 50 replicates per leaf species to be evenly split among five fungicide treatments (n=10), with a fully-crossed 3x5-factorial design for 21 days (Fig. 2 – Step 3; See **Appendix I**). Each replicate consisted of a 1 L glass beaker filled with 750 mL nutrient medium (Dang et al., 2005), 3 g microbial inoculum (wet weight i.e., of pre-conditioned leaves), 3 unconditioned leaf strips in mesh bags preventing the strips from sticking together and ensuring the accessibility of the leaf material for microorganisms, as well as the fungicide mixture. Experiments were conducted at 16 ± 1°C under continuous aeration, in darkness and medium renewal every 7 days (Fig. 2). At the end of the experiment, leaf litter decomposition rates were quantified as a functional endpoint, following Benfield (2007). Additionally, ergosterol content (as a proxy for fungal biomass; (Gessner, 2005) and bacterial density (Buesing, 2005) were measured to quantify microbial abundance as structural endpoints (see **Appendix I** for details).

Table 1. Information on the fungicide mixture components, their product names, manufacturers, active ingredient concentrations, nominal concentrations, and mode of action. Table taken from Appendix I.

Substance	Product name	Manufacturer	Active ingredient concentration	Nominal concentration (µg/L)	Mode of action
Azoxystrobin	Ortiva	Syngenta Agro	250 g/L	0; 0.5; 5; 50;500	Inhibition of mitochondrial respiration
Carbendazim	Derosol	Bayer crop science	600 g/kg	0; 0.5; 5; 50;500	Inhibition of mitosis and cell division
Cyprodinil	Chorus	Syngenta Agro	500 g/kg	0; 0.5; 5; 50;500	Inhibition of amino acid and protein synthesis
Quinoxyfen	Fortess 250	Dow Agro Science	250 g/L	0; 1; 10; 100;1000	Perturbation of signal transduction
Tebuconazole	Folicur	Bayer crop science	250 g/L	0; 0.5; 5; 50;500	Inhibition of sterol biosynthesis

Paper II - Effects of combined exposure to fungicides and nutrients on leaf-associated microbial communities with differing exposure history

The upstream land-use defined the exposure history of leaf-associated microbial communities (Fig. 3). Pristine streams surrounded by forest-dominated catchments (P; sites P1, P2 and P3) were chosen as sampling locations, as well as streams impacted by either wastewater discharge (W; sites W1, W2 and W3) or vineyard run-off (V; sites V1 and V2; severe draughts during autumn 2019 did not allow to assess V3; see details in **Appendix II**). Three independent semi-static bioassays were performed during April-May (sites P1, W1 and V1); July-August (sites P2, W2 and V2) and September-October (sites P3 and W3) 2019. Each assay followed a 3x4x4-factorial design with a duration of 21 days and included one community per exposure type (i.e., P-, W- and V-community; Fig. 3 – Step 1).

Black alder (*Alnus glutinosa* (L.) Gaertn.) leaves were collected in the same region in the preceding years (stored frozen -20 ºC until use) and deployed in mesh bags at the sampling sites. The leaves were colonised by the local community of microorganisms for 14 days (Fig. 3 - Step 2, **Appendix II**). In the laboratory, the same leaves were acclimatised and homogenised to prepare a microbial inocula for the exposure phase (Fig. 3 – Step 3 & 4, see details in **Appendix II**). The exposure phase was conducted by exposing microbial communities to increasing concentrations of a fungicide mixture (0-300 µg/L, same fungicides as in Table 1, see mixture details in **Appendix II**) crossed with four increasing nutrient concentrations (Fig. 3 – Step 5). The nutrient and fungicide concentrations were selected based on previous studies (Feckler et al., 2018; Zubrod et al., 2015). The nutrient medium composition largely followed Dang et al. (2005) but was adjusted in terms of NO_3-N (0.2, 2.0, 10.0 and 18.0 mg/L) and PO_4-P (0.02, 0.2, 1.0 and 1.8 mg/L) concentrations. In the following, these nutrient concentrations are referred to as very low, low, moderate and high. The fully crossed design resulted in 48 treatments, each replicated five times (see details in **Appendix II**). Each replicate consisted of 20 leaf discs (Ø 20 mm cut from frozen and uncolonised leaves, dried and weighted to the nearest 0.01 mg), 5 mL of inocula suspension, 1 mL of fungicide stock solution, and autoclaved nutrient medium (final volume of 50 mL) in sterilized 150-mL Erlenmeyer flasks. The flasks were closed with

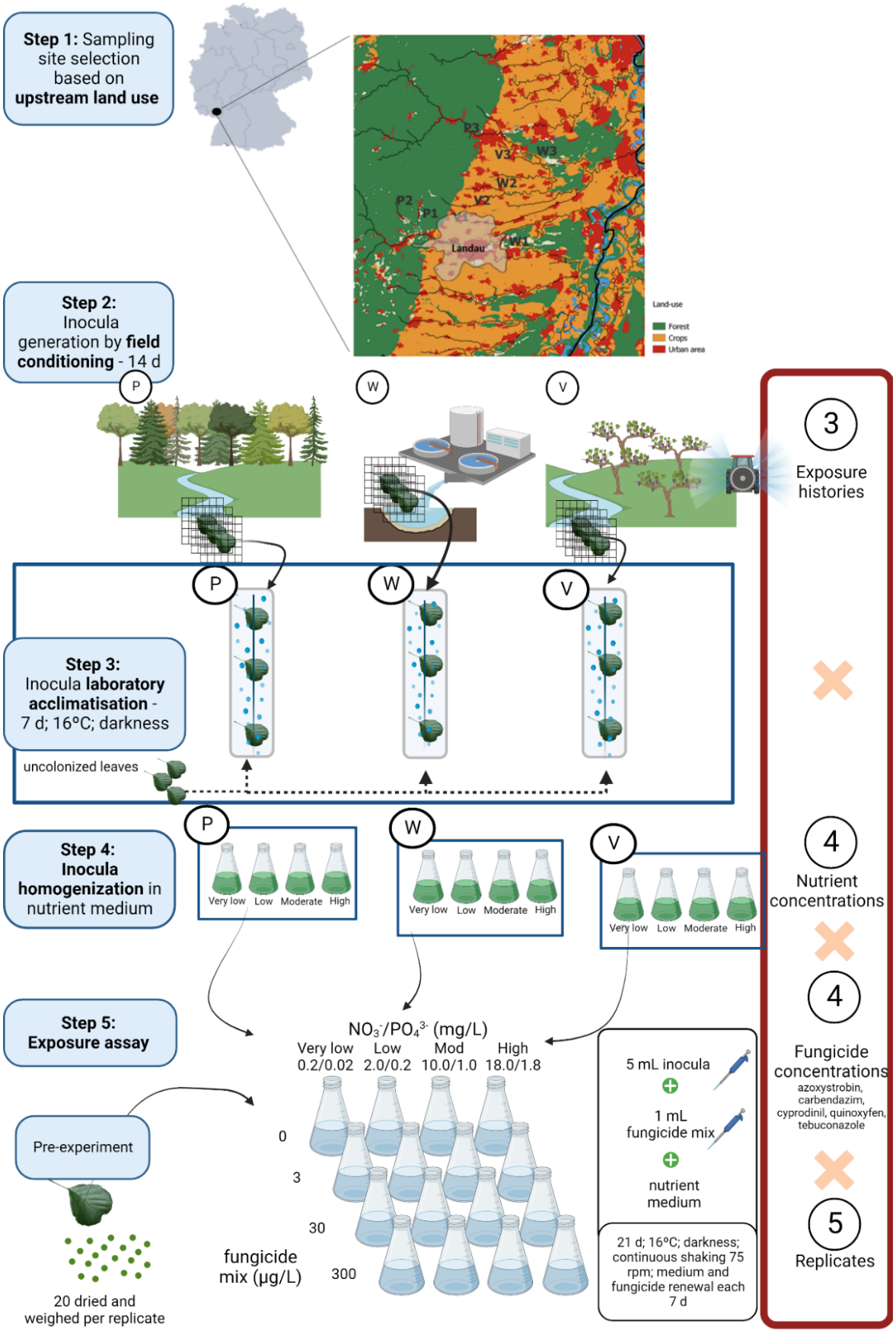

Figure 3. Schematic overview of the study design. Step 1: Selection of sampling sites based on upstream land-use. Step 2: Generating inocula from pristine (P) streams, or streams impacted by wastewater discharge (W) and vineyard run-off (V) by deploying alder leaves in the field for 14 days; Step 3: Inocula acclimatisation to laboratory conditions; leaves from each sampling site and uncolonised leaves are further microbially colonized for 7 day; Step 4: Inocula (leaves) homogenisation in nutrient media per exposure history and respective; Step 5: Exposure assay – the inocula prepared were used to microbially colonize leaf discs in Erlenmeyer flasks, while being exposed to increasing concentrations of nutrients and fungicides over 21 day, with media and fungicides being renewed every 7 day. Created with BioRender.com. Figure taken from Appendix II.

sterile culture cellucotton plugs allowing air exchange, kept at 16 ± 1 ºC in darkness under continuous orbital shaking at 75 rpm, while the nutrient medium together with the fungicide mixture was renewed every seven days (**Appendix II**).

At the end of the experiment, we measured microbially-mediated leaf litter decomposition (Benfield, 2007) and exoezyme activity (Baudy et al., 2021; DeForest, 2009) as a functional endpoints. Aditionally, the communities' structure was studied via fungal and bacterial abundance (Manerkar et al., 2008) and fungal community composition through next generation sequencing (NGS; Carl et al., 2022). See detailed information for methods in **Appendix II**.

Paper III - Microbial community exposure history and leaf species effects on *Gammarus fossarum*

Bottom-up effects on shredders were assessed by focusing on leaf-associated microbial communities with distinct exposure history (first factor) using previously studied sites in Paper II: one pristine site (P1 – mainly dominated by forest in the nature conservation area) and one site characterised by repeated fungicide exposure in viticulture (V2, without riparian vegetation; Fig. 4; Fernández et al., 2015; Schneeweiss et al., 2022). The remaining factors to be assessed referred to the leaf species (alder and beech and their mixture) and the *Gammarus* sex (male and female), in a 2x3x2-factorial design (n=20, Fig. 4). Black alder and European beech were selected to represent a low and high degree of recalcitrance, respectively (Artigas et al., 2012; Gulis, 2001; **Appendix III**).

Stream water from both sites (P and V; 25 L) was collected weekly and used for conditioning leaves of alder, beech, and their mixture, generating distinct leaf-associated microbial communities in separate 50-L stainless-steel channels, kept at 20 ± 1 ºC in darkness under permanent aeration inducing water movement for 14 days (Fig. 4 - Step 1). This step resulted in six food sources for *G. fossarum* during the feeding assay (Fig. 4 – Step 2). The conditioning step was repeated weekly to ensure the provisioning of food with comparable quality over the entire study duration, namely 21 days. *G. fossarum* were collected from the same P site and transported to the lab to be divided by diameter (1.3-2 mm; Franke, 1997) and sex (Fielding et al., 2003; Pascoe et al., 1995). *Gammarus* were kept in aerated test medium for 14 days and acclimatized to 20 ± 1 ºC in darkness while being fed with unconditioned alder leaves (see details **Appendix III**).

During the feeding assay, leaf discs from the food source prepared were cut and offered to the *Gammarus*. Each replicate consisted of a 250-mL glass beaker equipped with 2 cages (see Zubrod et al., 2015, Fig. 4 – Step 2) and filled with 250 mL test medium (SAM-5S; Borgmann, 1996; automatically renewed twice a day). Every seventh day, remaining leaf discs and faeces were retrieved and gammarids were translocated to a new beaker with fresh leaf discs. The remaining leaf discs and old medium were collected to determine feeding rate and faeces production (Zubrod et al., 2011). At the experiment termination, also surviving *Gammarus* (mortality did not

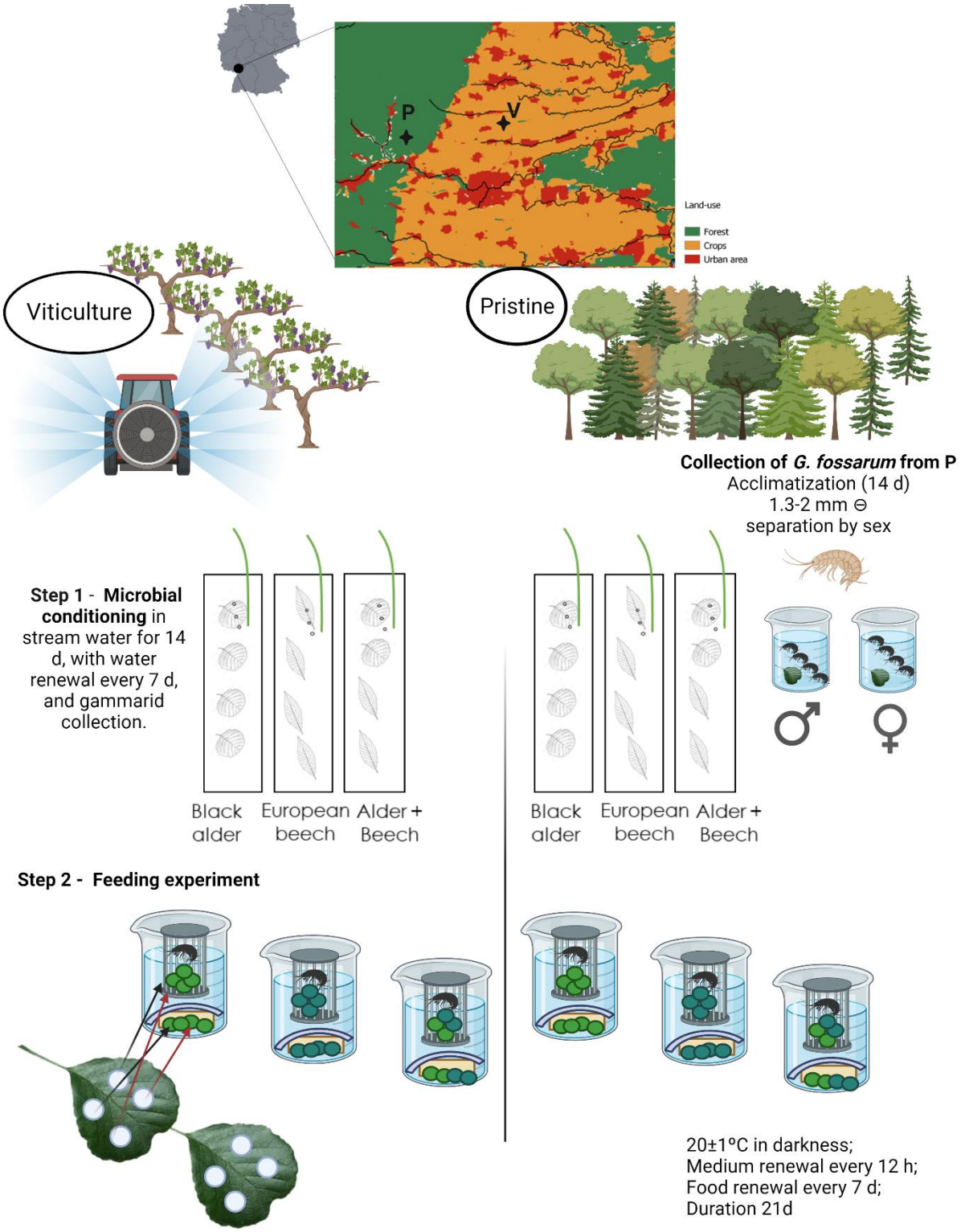

Figure 4. Schematic overview of the study design. Step 1: Preparation for the feeding experiment: generating inocula and collecting test organisms – sampling stream water and *Gammarus fossarum* from a near-natural stream (pristine, P- community). Simultaneously, a stream surrounded by viticulture (V- community) was sampled. In the laboratory, the stream water was used to microbially colonise alder and beech leaves or a mixture of both in stainless steel channels under continuous aeration (green lines). Gammarids were separated by diameter and sex and kept in aerated medium, while fed with alder leaves *ad libitum* during acclimatization (14 d). Step 2: 21 d feeding experiment with a 2x3x2-factorial design (n=40). Per replicate 8 discs (Ø=16 mm) were cut of leaves generated in step 1, here only exemplified for alder treatment. Four leaf discs of each leaf species combination were fed to each gammarid, and another 4 leaf discs were used to control for leaf mass loss (orange rectangle), separated by a watch glass (grey line). Created with BioRender.com. Figure taken from Appendix III.

exceed 5%) were shock frozen in liquid nitrogen and stored at −80 °C (see details **Appendix III**).

Leaf-associated microbial communities (used as food sources) were characterised by their exoenzyme activity (Baudy et al., 2021; DeForest, 2009) as a functional endpoint, and ten AH species composition as well as fungal and bacterial abundances (Manerkar et al., 2008) as structural endpoints. Additionally, responses of *Gammarus* to the food source were assessed by measuring their growth rate in terms of biomass increase, feeding rate and faeces production (Zubrod et al., 2011), as well as their energy reserves in the form of neutral lipid fatty acid (NLFA) profiles (Bligh & Dyer, 1959; Konschak et al., 2020; see detailed information **Appendix III**).

4. RESULTS AND DISCUSSION

4.1 Paper I - Effects of fungicides on leaf-associated microbial communities colonising different leaf species

Alder and maple were decomposed faster than beech in the absence of fungicides (Fig. 5; **Appendix I**). In the presence of fungicides, leaf litter decomposition, fungal biomass and partially bacterial density were negatively impacted for all leaf species (Fig.5; $p<0.05$, Table 2; **Appendix I**). For leaf litter decomposition, the interaction term of the factor “leaf species” and “fungicide” was non-significant ($p>0.9$; Table 2; **Appendix I**), pointing to a similar response pattern of leaf litter decomposition (decreasing) among leaf species with increasing fungicide concentrations. Nevertheless, relevant differences between leaf species can be found as the highest reductions in decomposition rates varied by a factor of two (12 vs 21 and 20% reduction for alder, maple, and beech, respectively, between control and 3000 µg/L; **Appendix I**). The decreases found in leaf litter decomposition support the negative impacts of the fungicides and tended to increase for leaf species with less favourable traits. These combined effects were particularly pronounced for fungal biomass, measured as ergosterol (Table 3, **Appendix I**).

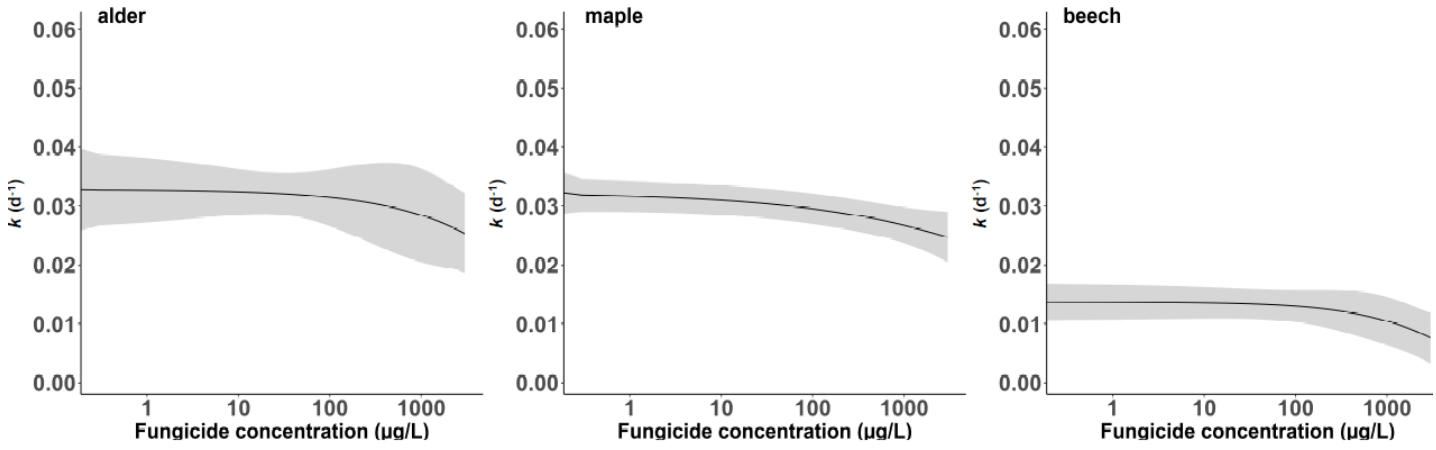

Figure 5. Concentration-response models (solid lines; shaded lines indicating corresponding 95% CIs; n = 10) for the leaf litter decomposition rate, *k* (d^{-1}), as a function of the total fungicide concentration for the different leaf species alder, maple and beech. Figure taken from Appendix I.

Fungal biomass was lower in alder leaves when compared to maple and beech (Table 3). This observation may be explained as the fungal biomass is a group

measure, which does not take in account the AH single species composition and therefore the potential replacement of less efficient fungal species by species with a higher decomposition efficiency (Baudy et al., 2021a). Moreover, as alder offers close to optimal conditions for microbial communities' growth (Artigas et al., 2012), the alder-associated fungal biomass might have already peaked before the termination of the experiment (Baldy et al., 1995). On the contrary, the maximum of ergosterol for maple and beech may not yet have been reached at test termination (**Appendix I**). Bacterial density results, on the other hand, have not shown a consistent pattern between leaf species and increasing concentrations of fungicides, which likely supports their minor but not negligible contribution to leaf litter decomposition (Hieber & Gessner 2002).

Despite significant changes in decomposition rates not being found for alder compared to control in our study, significant changes of this function were detected for alder in a previous study (Zubrod et al., 2015). At the same time, the effect size observed (~20%) for alder at 3000 µg/L is in accordance with Zubrod et al. (2015).

Table 2. Output for statistical analysis of the rank-based ANOVA. Degrees of freedom (Df); sum of squares (Sum Sq); mean squares (Mean Sq). P-values printed bold indicate statistical significance. Table taken from Appendix I.

Enpoint	Source of variation	Df	Sum Sq	Mean Sq	F value	P-value
Leaf litter decomposition rate	**Leaf species**	2	0.0107	0.0054	66.394	**p < 0.001**
	Fungicide	4	0.0009	0.0002	2.824	**0.027**
	Leaf species x fungicide	8	0.0002	0.0001	0.387	0.926
	Residuals	135	0.0108	0.0001		
Fungal biomass (ergosterol)	**Leaf species**	2	396.2	198.1	21.118	**p < 0.001**
	Fungicide	4	2751.7	687.9	73.341	**p < 0.001**
	Leaf species x fungicide	8	290.5	36.3	3.872	**p < 0.001**
	Residuals	135	1266.3	9.4		
Bacterial density	**Leaf species**	2	1.25×10^{18}	6.26×10^{17}	31.205	**p < 0.001**
	Fungicide	4	2.10×10^{17}	5.25×10^{16}	2.618	**0.038**
	Leaf species x fungicide	8	1.37×10^{17}	1.71×10^{16}	0.855	0.557
	Residuals	130	2.61×10^{18}	2.01×10^{16}		

For the other leaf species, the decomposition rate was affected similarly between maple and beech, with effect size being twice as high when compared to alder. Maple and beech showed a non-significant reduction in the leaf litter decomposition rate of up to ~20% at the two highest fungicide concentrations (300-3000 µg/L). Changes in fungal biomass support this pattern (see also Zubrod et al.,

2015), with a lower reduction of the ergosterol concentration on alder relative to beech or maple among fungicide treatments (**Appendix I**). Additionally, an interaction of "leaf species" and "fungicide" was only found for fungal biomass, suggesting a non-additive effect of both factors. These observations suggest that alder leaves traits' (high nutrient levels and low recalcitrance) enable microbial communities to acquire leaf-bound energy more easily to withstand potential effects induced by fungicide exposure (Solé et al., 2012).

Table 3. Bacterial density, as number of cells per mg leaf dry weight, and ergosterol concentration, as µg per mg of leaf dry weight, of different leaf species (alder, maple, and beech) ± 95% CIs., for the increasing fungicide concentrations. Table taken from Appendix I.

Leaf species	Fungicide concentration (µg/L)	Bacterial density (number of cells 10^8/mg leaf dw)	Ergosterol concentration (µg/mg leaf dw)
alder	0	3.04 ± 0.68	8.40 ± 1.17
	3	3.33 ± 0.44	6.55 ± 1.07
	30	2.08 ± 0.21	6.90 ± 1.10
	300	2.48 ± 0.40	4.86 ± 0.92
	3000	2.40 ± 0.29	0.56 ± 0.15
maple	0	3.49 ± 0.27	14.11 ± 0.80
	3	4.60 ± 0.79	14.79 ± 1.00
	30	3.90 ± 0.64	11.03 ± 0.99
	300	2.56 ± 0.19	5.90 ± 0.82
	3000	3.52 ± 0.28	0.82 ± 0.06
beech	0	1.33 ± 0.10	12.70 ± 0.75
	3	1.53 ± 0.24	11.82 ± 1.20
	30	1.67 ± 0.19	11.54 ± 1.03
	300	0.88 ± 0.10	3.87 ± 0.43
	3000	1.51 ± 0.08	0.14 ± 0.04

Despite statistically non-significant (Table 2), this interpretation is backed by fungal biomass being more reduced under fungicide exposure on the most recalcitrant and least nutrient-rich leaf species (namely beech) – an observation made by Artigas et al. (2012) and supported by the present study. In their study, the presence of 30 µg tebuconazole/L induced a 60% higher reduction in fungal biomass associated with more recalcitrant black poplar (*Populus nigra* L.) relative to alder. The differences in fungicide effects between maple and alder, both with comparable decomposition rates, are potentially related to maple having a comparatively smooth surface on both leaf sides which makes the colonisation and penetration by fungi more challenging (Kearns & Bärlocher, 2008). Consequently, fungal propagules are exposed to fungicides for a

longer period, which increases the effects on leaf litter decomposition. On alder, however, the propagules can quickly attach and grow into the leaf (Kearns & Bärlocher, 2008), which may provide protection, reducing the fungicide exposure. Moreover, some fungicides only act on the propagules of fungi and not on growing mycelium (Escudero-Leyva et al., 2022). Even though these findings may seem of little relevance, the combination of leaf traits (nutrients, recalcitrant substances, surface) with fungicide stress may have contributed to the more pronounced fungicide effect at higher concentrations in beech and maple leaves (**Appendix I**).

4.2 Paper II - Effects of combined exposure to fungicides and nutrients on leaf-associated microbial communities with differing exposure history

Effects of fungicides on microbial communities with differing exposure histories.

Increasing fungicide concentrations did not significantly affect leaf litter decomposition, independent of the nutrient concentration used (Fig. 6, Table 4, **Appendix II**), or the relative investment in degrading recalcitrant carbon (i.e., ratio of oxidase per total hydrolase enzymatic activity). Instead, a positive effect on the leaf litter decomposition was observed for communities originally sampled from P- and W-streams at 30 and 300 µg/L (see **Appendix II** for details). However, the same pattern was not found for microbial community composition, also reported by e.g., Feckler et al., 2018; Fernández et al., 2015. If at low fungicide concentrations (3 and 30 µg/L), bacterial and fungal abundance were not affected, at 300 µg/L, fungicides had a negative impact (up to 60%) on the fungal abundance.

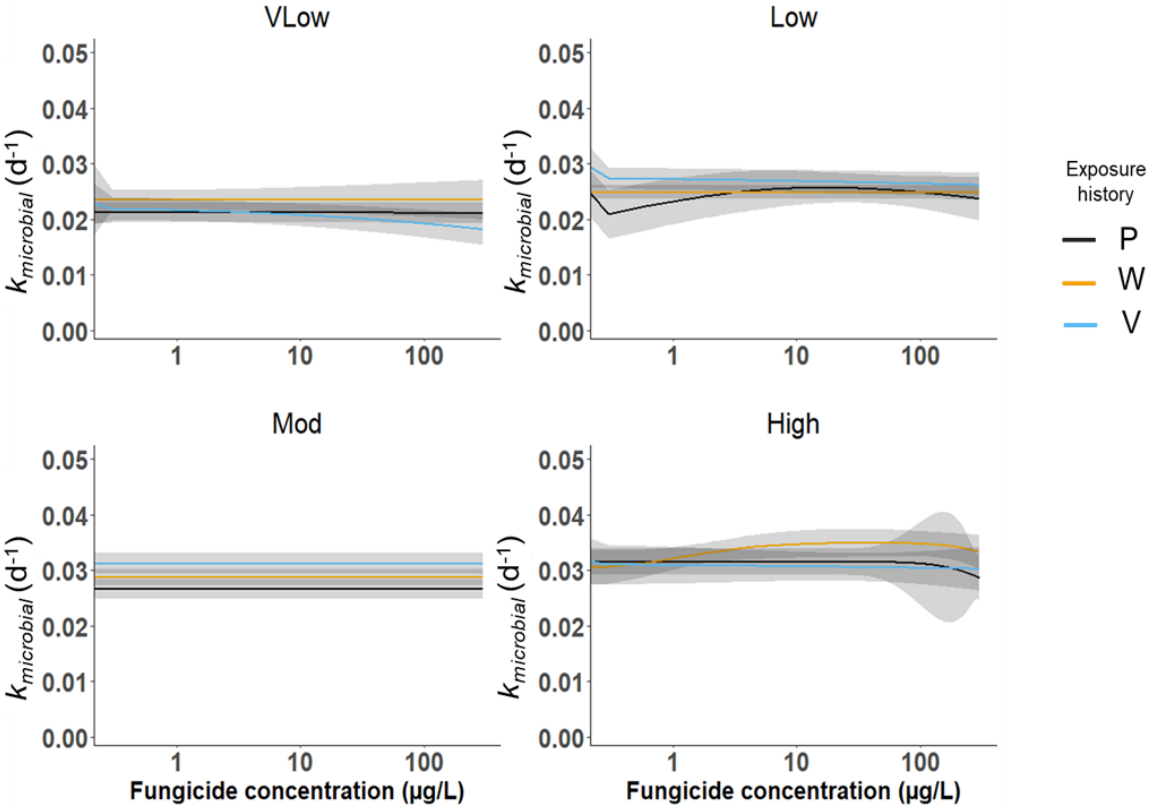

Figure 6. Dose-response models for the microbial breakdown rate ($k_{microbial}$ (d^{-1})) as a function of the total fungicide concentration, displayed separately for the four different nutrient levels (VLow-very low, Low, Mod - moderate and High). Shaded lines indicating corresponding 95% confidence bands (n = 5). P: pristine; W: wastewater; V: vineyard run-off. Figure taken from Appendix II.

Table 4. Output for statistical analysis, aligned ranks transformation ANOVA of leaf microbial decomposition, bacterial and fungal DNA operon copies (for respective relevant post-hoc testing see paper I); ANOVA run in univariate data (Recalcitrance ratio); PERMANOVA run in multivariate data (community composition). Df, degrees of freedom; Df res, residual degrees of freedom for each model: F value, ratio of variances; SE, standard error of the estimate SS, sum of squares; p-values printed in bold indicate statistical significance. Table taken from Appendix II.

Endpoint	Method	source of variation	Df		Df res	F value	p-value
Leaf litter decomposition	Aligned ranks transformation ANOVA	Fungicide	3		592	0.3670	0.7769
		Nutrient	**3**		**592**	**70.9385**	**<0.0001**
		History	**2**		**592**	**6.5923**	**0.0010**
		Fungicide x Nutrient	9		592	1.4461	0.1649
		Fungicide x History	6		592	1.1515	0.3309
		Nutrient x History	**6**		**592**	**3.1005**	**0.0053**
		Fungicide x Nutrient x History	18		592	0.2686	0.9990
Bacteria	Aligned ranks transformation ANOVA	**Fungicide**	**3**		**336**	**8.2042**	**<.0001**
		Nutrient	3		336	1.8397	0.1397
		History	**2**		**336**	**4.0090**	**0.0190**
		Fungicide x Nutrient	9		336	0.8542	0.5667
		Fungicide x History	6		336	0.2029	0.9758
		Nutrient x History	**6**		**336**	**3.0591**	**0.0063**
		Fungicide x Nutrient x History	18		336	1.1867	0.2696
Fungi	Aligned ranks transformation ANOVA	**Fungicide**	**3**		**336**	**7.4994**	**<.0001**
		Nutrient	3		336	1.8887	0.1312
		History	**2**		**336**	**3.0893**	**0.0468**
		Fungicide x Nutrient	9		336	1.0137	0.4286
		Fungicide x History	6		336	0.2342	0.9652
		Nutrient x History	**6**		**336**	**4.2557**	**0.0003**
		Fungicide x Nutrient x History	18		336	1.3180	0.1734
		source of variation	**Df**	**SS**	**Df res**	**F value**	**p.value**
Recalcitrance ratio	ANOVA	Fungicide	1	0.0000003	0.0000003	0.003	0.958
	PERMANOVA	Nutrient	3	0.0001539	0.0000513	0.483	0.697
Community composition		History	2	0.0002003	0.0001001	0.943	0.403
		Fungicide x History	2	0.0001189	0.0000595	0.560	0.579
		Nutrient x History	6	0.0001472	0.0000245	0.231	0.962
		Fungicide x Nutrient	3	0.0000523	0.0000174	0.164	0.919
		Fungicide x Nutrient x History	6	0.0002063	0.0000344	0.324	0.918
		Residuals	24	0.0025488	0.0001062		
Community composition	PERMANOVA	**Fungicide**	**1**	**2.7383**	**0.12758**	**11.145**	**0.001**
		Nutrient	**2**	**0.7115**	**0.03315**	**1.4479**	**0.034**
		History	**2**	**1.3408**	**0.06247**	**2.7287**	**0.001**
		Fungicide x Nutrient	2	0.5718	0.02664	1.1636	0.208
		Fungicide x History	**2**	**0.7535**	**0.03511**	**1.5334**	**0.018**
		Nutrient x History	4	1.1051	0.05149	1.1245	0.197
		Fungicide x Nutrient x History	4	0.9741	0.04538	0.9911	0.485
		Residual	54	13.2675	0.61817		
		Total	71	21.4626	1		

The negative impact on fungal abundance was independent of the exposure history or nutrient concentration ($p<0.05$; Table 4; **Appendix II**). Moreover, independent of the fungicide concentration, the bacterial and fungal abundances were consistently lower

in the V-communities compared to the equivalent treatment in the W- and P-communities, however not statistically significant (**Appendix II**).

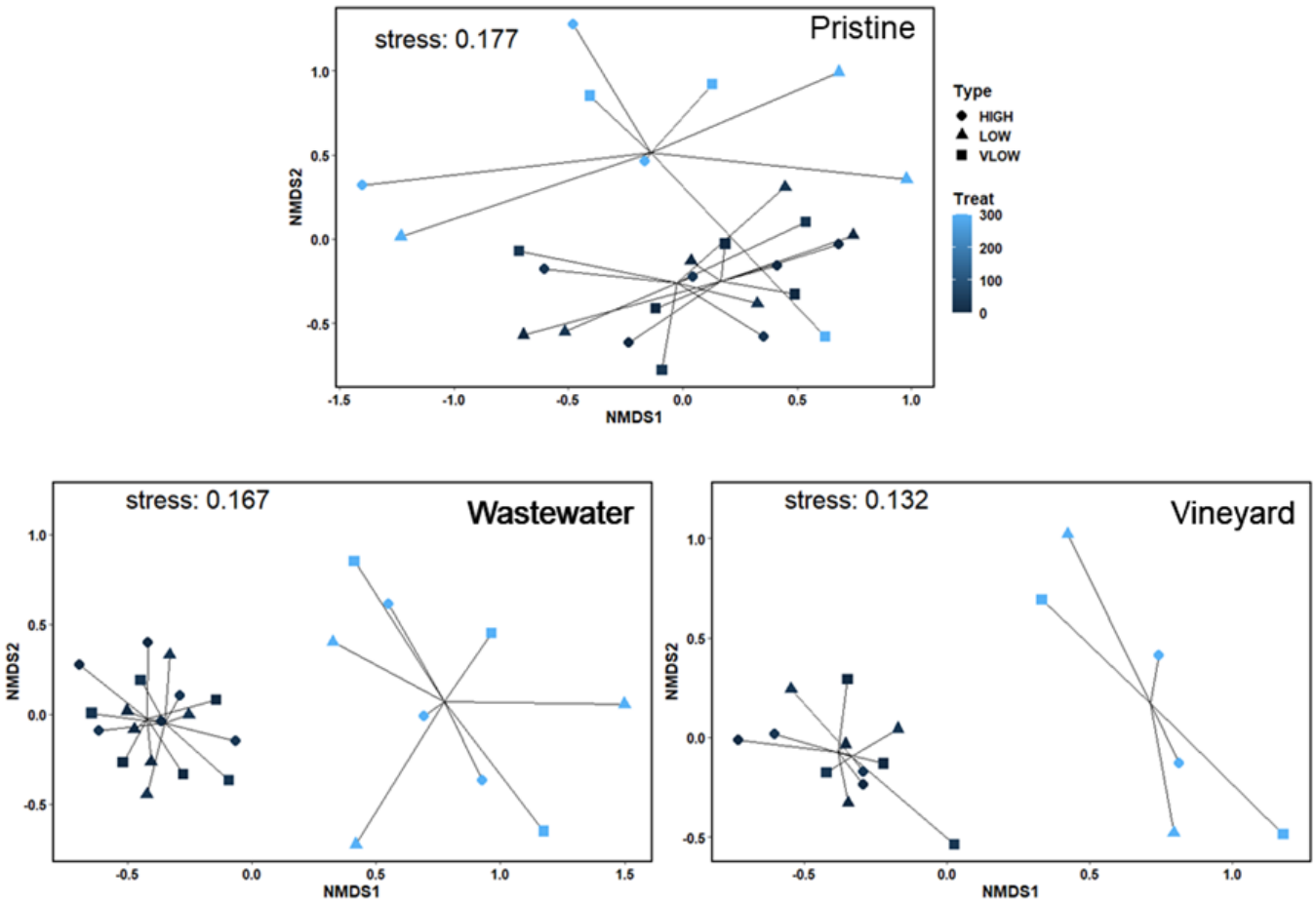

Figure 7. Non-metric multidimensional scaling (NMDS) plots for leaf-associated aquatic hyphomycete communities originating from streams with differing land-use in their catchments (Pristine, Wastewater treatment plants, Vineyard). Nutrient levels are indicated by symbols: very low= squares, low= triangles, high = circles. Colours indicate fungicide concentrations: 0 µg/L and 30 µg/L = dark blue, 300 µg/L = light blue. Spider webs connect the samples of each treatment at their respective group centroid. The stress value is provided as a measure of "goodness-of-fit" for NMDS, with a reasonable fit indicated when below 0.2 (Clarke, 1993). Figure taken from Appendix II.

In addition to the impacts on fungal abundances, a similar pattern was observed for fungal community composition (Fig. 7). Controls and treatments with lower fungicide concentrations (<30 µg/L) had similar community composition, whereas in higher fungicide concentration the fungal species composition differed substantially (p=0.001; Table 4; Fig. 7). Differences found in species composition were dependent of the nutrient levels and exposure history (p=0.001; Table 4; **Appendix II**). These results partially contradict the existence of the link between fungal community structure

and function (Hooper et al., 2012). Instead, the results point towards functional stability reached due to functional similarity and the dominance of tolerant and simultaneously more efficient AH species in leaf litter decomposition (Ferreira & Chauvet, 2012; Pascoal et al., 2005), despite community shifts (reviewed in Feckler & Bundschuh, 2020). This assumption is supported by our community composition data, where the tolerant genus *Tetracladium* with a higher leaf litter decomposition efficiency (e.g., Andrade et al., 2016; Duarte et al., 2006; Zubrod et al., 2015; Zubrod et al., 2015) was more frequent at high fungicide concentration, independent of exposure history (**Appendix II**). While other fungal species considered tolerant have also become more frequent with increasing fungicide concentrations, knowledge on their traits is limited and partly contradicting hampering a mechanistic interpretation (e.g., Bundschuh et al., 2011; Pascoal et al., 2005). For example, Bundschuh et al. (2011) reported that *F. curvula* was less abundant at higher fungicide concentrations while more present under control conditions. Contrarily, we found this species most frequently in the presence of fungicides suggesting phenotypic plasticity (e.g.,Quainoo et al., 2016).

Our findings therefore support the principle of stable functioning being mediated by the dominance of highly efficient decomposers. These results are supported by earlier studies (reviewed in Feckler & Bundschuh 2020), pointing to a maintained leaf litter decomposition function when the microbial community is dominated by a few species with superior traits compensating biodiversity loss (Dangles & Malmqvist, 2004).

Effects of nutrients on microbial communities with differing exposure histories.

Increasing nutrient concentrations were significantly favourable for leaf litter decomposition ($p<0.0001$; Table 4; Fig. 6), especially at moderate and high nutrient levels, while the effect strength depended on the exposure history ($p=0.005$; Table 6; **Appendix II**). The effect of moderate and high nutrient levels may be explained by the dynamic energy budget theory (Kooijman, 2000), in which microbial growth and function is supported by the ease of accessing nutrients from the medium as more energy is available for producing exoenzymes needed for leaf litter decomposition (Bärlocher & Corkum, 2003). Similar findings have been reported by Feckler et al. (2018) supporting our assumption: higher leaf litter decomposition in treatments with higher nutrient availability (see also Pascoal & Cássio, 2004; Suberkropp et al., 2010). Consequently, it is likely that in ecosystems with higher nutrient inputs, changes in the

microbial function due to stress exposure being less pronounced due to “extra” energy from the available nutrients (see Rossi et al., 2018 but also see Fernández et al., 2016). Nevertheless, community structure was significantly affected by exposure history, with P-communities being characterised by up to 20-fold higher bacterial and fungal abundances compared to W- and V-communities within the same nutrient level (see **Appendix II**). Whereas leaf litter decomposition was slightly higher in W- compared to P-communities (~15%; $p<0.003$, **Appendix II**), while the function of P-communities was 40% higher than V-communities ($p<0.01$; **Appendix II**). These opposing observations may be an experimental artefact since changes in the fungal community composition and consequently its composition in terms of functional traits are not accounted for the proxies used for bacterial and fungal microbial abundances (Englert et al., 2015; Rossi et al., 2018). It is likely that microbes characterised by a high leaf litter decomposition efficiency dominate over those with a lower efficiency capable of maintaining the function (e.g., Reiss et al., 2010).

Combining chemical stressors and exposure history.

Overall, we found changes in the community structure at high fungicide exposures (300 µg/L) across all exposure histories. Additionally, the factors “fungicides” or “history” did not affect the degradation of recalcitrant carbon by microbial communities, but the increasing levels of nutrients tended to buffer the non-significant fungicide-induced effects on leaf litter decomposition. However, we expected more pronounced effects of the fungicides on P- communities compared to the pre-exposed W- and V- communities. The presence of some tolerant species, such as *T. marchalianum*, also in P-communities, may explain this observation. These results (high variability and non-consistent patterns) point towards a significant role of local communities and colonisation dynamics (Mora-Gómez et al., 2016). Therefore, the impacts of these last factors should be individually expanded in further research, also including other relevant factors not assessed here as season.

4.3 Paper III - Microbial community exposure history and leaf species effects on *Gammarus fossarum*

Responses of sexes of *Gammarus* to different food qualities.

Chemical signals from fungi and bacteria can attract shredders, promoting their feeding activity on colonised leaf material (Lange et al., 2005). However, the role of bacteria in gammarids' nutrition remains largely ignored. Unfortunately, our results on bacterial abundance did not provide a clear pattern and consequently any interpretation of bacteria's role would speculative (Table 5; **Appendix III**). Moreover, the overall fungal abundance (operon copies) in this study was up to 40 % lower but statistically insignificant in treatments where beech was present compared to alder only (Table 5; **Appendix III**). This observation is partially in accordance with the findings of Paper I, where leaf species with deviating traits (e.g., alder vs beech) are colonised by structurally different microbial communities (**Appendix I**). Both suggest a likely lower nutritional value of the food sources for gammarids when beech leaves are present. However, literature rather proposes a shredders' preference for certain AH species (i.e., AH community is considered the main driver of leaf litter palatability for shedders; Arsuffi & Suberkropp, 1984). Indeed, in the present study the AH community composition (evaluated by ten representative AH species) varied significantly between P- and V-communities ("exposure history"; p=0.004), among "leaf species" (p=0.001) and an interaction between thereof was observed (p=0.048; Fig. 8, **Appendix III**).

Table 5. Mean (with 95 % confidence intervals; 10^8 operon copies/mg leaf dw; n=3, fungal and bacterial operon copies of microbial communities colonising the leaves used as food for *G. fossarum* during the 21-d lasting feeding assay. P: pristine; V: vineyard run-off. Taken from Appendix III.

Organism group	Endpoint	Treatment																		
		alder-P			alder-V			alder-beech-P			alder-beech-V			beech-P			beech-V		
Fungi	Operon copies/ mg leaf dw	4.66	±	3.30	6.78	±	6.73	5.33	±	3.6	3.44	±	3.47	3.76	±	3.43	3.56	±	3.2
Bacteria		0.51	±	0.92	1.72	±	2.03	1.67	±	1.22	0.59	±	0.59	0.71	±	0.73	0.58	±	0.56

Species such as *Alatospora acuminata* and *Flagellospora curvula* were present in all treatments but were significantly reduced (~70%) on beech leaves conditioned by the V- relative to the P-community (**Appendix III**). These results are partially in

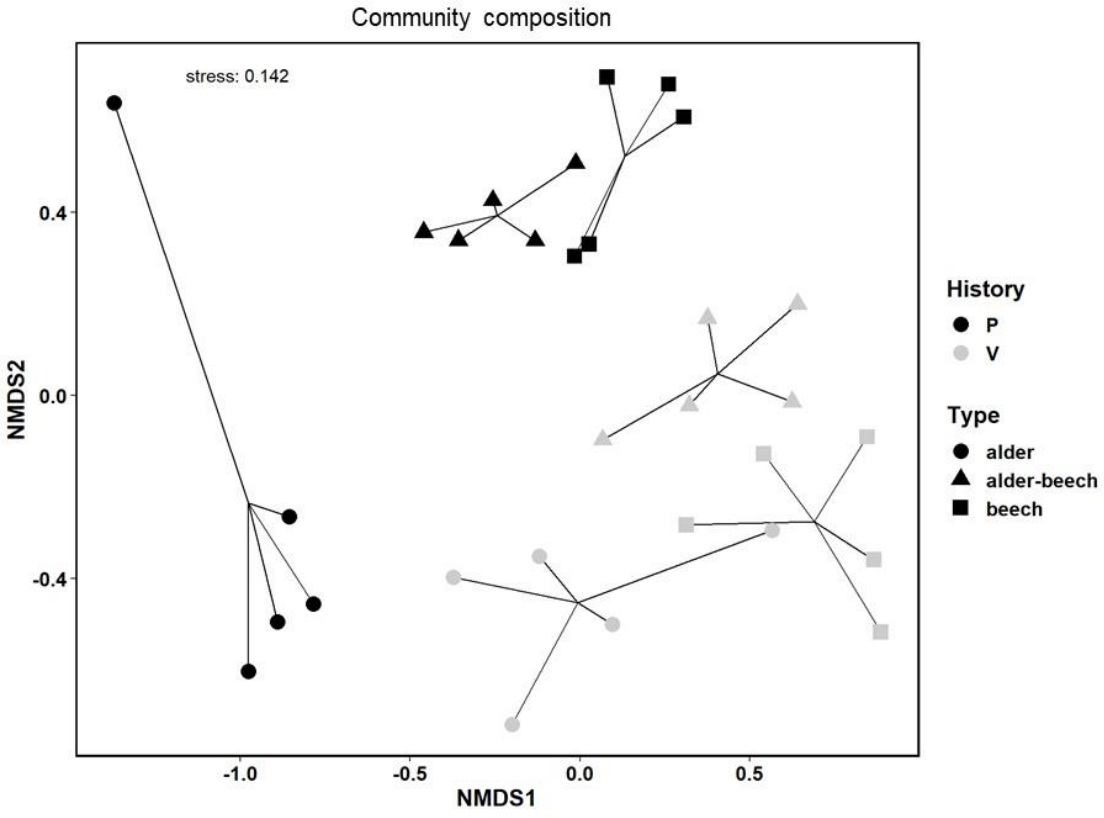

Figure 8. Non-metric multidimensional scaling (NMDS) plot for leaf-associated aquatic hyphomycete communities. Leaf species are indicated by symbols (alder = circles, beech = squares, the mixture of both = triangles). Colours indicate the source of microbial inocula: pristine stream water (P) = black and vineyard run-off stream water (V) = grey. Spider webs connect the samples of each treatment at their respective group centroid. The stress value is provided as a measure of "goodness-of-fit" for NMDS, with a reasonable fit indicated when below 0.2 (Clarke,1993). Figure taken from Appendix III.

accordance with the changes found for V-communities in Paper II (**Appendix II**). Nevertheless, in the present study no relation between shedders' preference (Fig. 9) and fungal biomass or enzymatic production (table 5; **Appendix III**) could be established (Suberkropp et al., 1983). Instead, it is likely that the individual AH species traits, such as secondary metabolites (Arsuffi & Suberkropp, 1984), or mycelia's glyceride or FA content (Arce Funck et al., 2015; Cargill et al., 1985) are motivating shedders' preferences for specific fungal species. In this context and independent of the leaf species, AH species considered more palatable (e.g., *A. acuminata, F. curvula*; Suberkropp et al., 1983; Arsuffi & Suberkropp, 1989) had equally high or higher biomasses on leaves conditioned by the P- relative to the V-community. These AH species are also expected to be more nutritional (Arce Funck et al., 2015; Rong et al., 1995) for *Gammarus.* In contrast, species such as *Tetracladium marchalianum* or

Tricladium angulatum, also expected to be less nutritional, were absent or had a lower biomass on leaves conditioned by the P-community compared to leaves conditioned by the V-community (as in e.g., Arsuffi & Suberkropp, 1989; Bärlocher, 1973; Gonçalves et al., 2014). This pattern suggests that more tolerant species, eventually dominate stressed fungal communities (e.g., *T. marchalianum;* Solé et al., 2008; Bundschuh et al., 2011). Moreover, patterns between AH species composition and different leaf species were not consistent. Consequently, a generalizable pattern of AH community composition among substrates or the origin of the microbial inoculum is not abstractable.

The different leaf species with different palatability described above should have had an impact on G*ammarus*' physiology. *Gammarus'* growth rate was significantly impacted by the leaf species (p=0.001) and showed a significant interaction of leaf species and the sex (p=0.005; **Appendix III**). Based on Gammarus' growth (Fig. 9), both sexes did not perform well when fed with beech only, a potential consequence of its higher recalcitrance and conditioning with less nutritional AH species (**Appendix I & III**). Moreover, males and females showed different growth patterns despite the partially high variability within treatments. Males and females grew faster, up to 60%, when feeding on alder and the mixture of both leaf species, compared to when feeding on beech, a pattern independent of the exposure history (**Appendix III**). Additionally, the feeding rate of females was slightly (5-30%) but consistently and significantly higher than that of males (p=0.048). Despite female feeding rate being higher than that of males, females produced less faces compared to males (~10-20% less production of faeces by females; Fig. 9 c & f; **Appendix III**). Faeces production was also higher when gammarids were feeding on the mixture of both leaf species, independent of sex and source of the microbial inoculum, which may be a consequence of a promoted feeding rate partially observed in this treatment (Fig. 9 b & e; **Appendix III**).

These results point towards different feeding preferences between males and females, which may be explained by sex-specific requirements and life history strategies. Male *Gammarus* live longer and have larger sizes than females, aiming to increase their competitiveness and support mate-guarding (Pöckl, 1992; Pöckl et al., 2003; Pöckl & Humpesch, 1990). Thus, males strive for resources optimising their growth. Addtionally to having the lowest feeding rate, males still grew faster (i.e., fed with alder), indicating an efficient use of high-quality leaf litter colonised by an AH

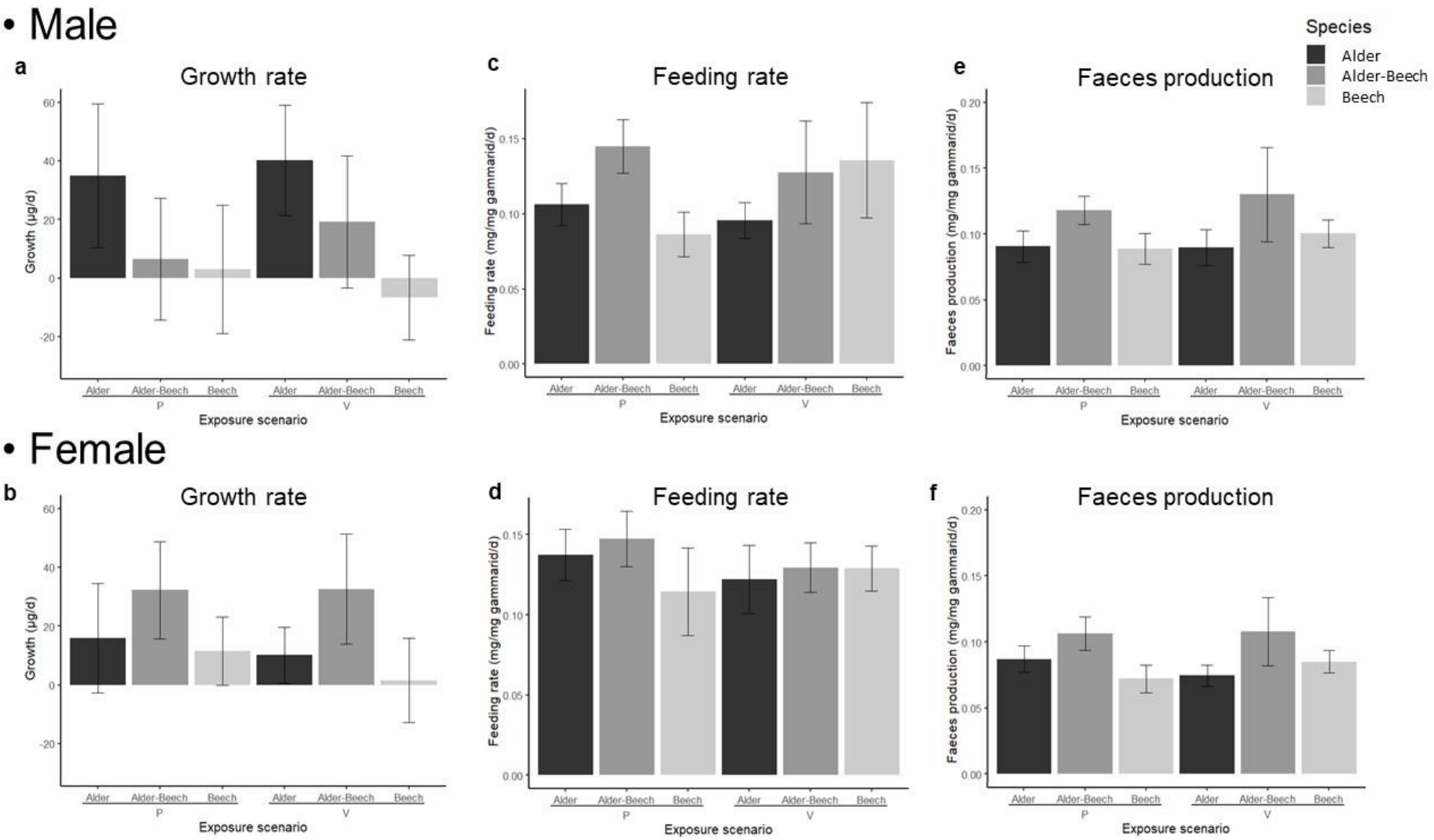

Figure 9. Mean (± 95% confidence intervals, n=20) a), b) growth rate as µg biomass gain/day, c), d) feeding rate as mg leaf material/mg gammarid/day, e), f) faeces production as mg faeces/mg gammarid/day of male and female gammarids, respectively, consuming alder (black), beech (light grey) or their mixture (dark grey) colonized by microbes with distinct exposure histories: P pristine; V vineyard. Figure taken from Appendix III.

community of presumably high nutritional quality. When beech leaves are introduced in the mixture, the food quality decreases leading to a higher feeding rate but lower growth of males. This observation suggests compensatory feeding, a mechanism by which organisms consume higher amounts of low-quality food to meet their nutritional requirements (Feckler et al., 2015; Rasmussen et al., 2012). NLFA profiles of male gammarids showed no significant differences among treatments (**Appendix III**). However, male gammarids exclusively feeding on beech had strongly reduced highly unsaturated (essential) FAs, such as ALA and EPA. Furthermore, the same pattern of NLFA profile was not observed with *Gammarus* fed on the mixture of both leaf species, supporting the assumption that alder may compensate for lower food quality of beech leaves. While these changes suggest implications in the physiology of the organisms, the reliability of the observed trends needs further support by follow-up experiments including data on female gammarids. Moreover, the shift to laboratory conditions and potentially lower quality food affected the gammarids, which had overall lower NLFAs' concentration compared to individuals from the start of the bioassay. It is likely that the not only gammarids supplement their dietary needs with other sources in the field (e.g., algae; Guo et al., 2016, 2018).

On the other land, females' strategy is to increase their size to enhance fecundity and carry eggs (Pöckl, 1990, 1992), with the latter also affecting their mobility and thus ability to exploit food resources (Lewis & Loch-Mally, 2010). We, consequently, assume females will constantly feed on any leaf species available to survive and wait for better conditions supporting growth, moulting and brood development. Earlier studies support our assumptions; Bakkar et al. (2017) demonstrated that male and female sesarmid crabs produced faeces with a different chemical signature when feeding on mangrove leaves, suggesting a sex-specific digestive process. Additionally, females may have evolved to use a mixed quality of food due to competitive nature behaviour (e.g., cannibalism as food preference over sex, (Dick et al., 1990; Dick, 1995; Ironside et al., 2019; Ward, 1983; Ward & Porter, 1993) and size advantage of males. Which is reflected in the present study by the efficient use of recalcitrant leaves, however this assumption needs further verification. Our results show a not straightforward relation from male to female responses, and thus any extrapolation (commonly used in previous studies due to reduced intra-treatment variability; Pascoe et al., 1995; Fielding et al., 2003) needs particular attention because of their relevance for population development. Overall, the present

study suggests that the leaf species identity, and thus the substrate on which the microbial communities grow, has a larger impact on the physiology of the next trophic level (i.e., the shredders) than the microbial community as such. As this observation is based on a limited number of community history replicates (i.e., one P-community and one V-community), its general applicability needs further scrutiny.

5. CONCLUSIONS

In this thesis, we tried to evaluate the effects of a common mixture of fungicides on leaf-associated microbial communities colonising different leaf species (i.e., substrate; **Appendix I**). Moreover, we tried to increase our understanding on the effects of different stressors, such as fungicides and nutrients, which have been frequently tested in previous studies, with expansion of field replication on exposure history (i.e., land-use) of leaf-associated microbial communities (**Appendix II**). Finally, we assessed if primary consumers feeding and development can be affected by food sources with different quality (i.e., leaf-associated microbial communities with different exposure history and colonising different leaf species; **Appendix III**).

More favourable traits (higher nutrient content and lower recalcitrance levels) of certain leaf species, such as black alder, enabled leaf-associated microorganisms to acquire leaf-bound energy and more easily resist the effects induced by fungicide exposure and thus being able to maintain the leaf-litter decomposition function (**Appendix I**). However, our research also shows the need to extend the knowledge on how leaf species' traits interact with stressors or other factors on the function and structure of microbial communities (**Appendix I & III**). The latter is particularly relevant as over the last decades and all across Europe, alder trees are being replaced in riparian zones. This replacement is happening due to different causes, such as habitat exploitation and pathogen infections, which will become more and more frequent. Consequently, changes in the composition of tree species along riverbanks are more expected. These changes in tree composition can either further diversify the leaf litter input into streams, due to the appearance of new tree species, or narrow down leaf litter diversity. In both cases, leaf litter susceptibility to be decomposed and used as a food source for shredder organism can suffer changes (**Appendix III**).

Secondly, increasing fungicide concentrations and exposure history did not affect leaf litter decomposition. Whereas increasing nutrient levels, tended to buffer the non-significant fungicide-induced effects on the function as it supports the microbial growth and its function (more energy for exoenzyme production). Moreover, substantial changes were found on the fungal community composition at environmentally relevant fungicide concentrations. Our results support the principle of species dominance, with highly efficient decomposers maintaining leaf litter decomposition function (functional stability); possibly at the expense of other functions provided by fungi (e.g., increase

palatability for shredders; **Appendix II**). These changes at the fungal community composition level combined with the lack of alterations at the functional level (i.e., leaf litter decomposition), raises potential concerns as in many cases only functional endpoints are used to assess the impact on the environment while structural changes remain unnoticed. This is an important subject as aquatic fungi have a key role in ecosystems, regulating aquatic food webs in a bottom-up direction (**Appendix II & III**). The fungal species considered more tolerant and efficient in leaf-litter decomposition are often rejected and not as nutritional for shredders, potentially affecting their development (**Appendix II & III**). Additionally, due to the high variability and non-consistent patterns found among the studied communities likely explained by different the sampling season and the respective naturally differing enzyme activities (Bastias et al., 2022), future research should be conducted. In this context, to further assess local (field) communities, potential community colonisation dynamics role and individual fungal traits will expand our mechanistic understanding of leaf-associated communities' response to multiple stress scenarios.

Finally, leaf species identity has a higher impact on the physiology of shredder invertebrate *G. fossarum*, relative to the community colonising the leaf material (**Appendix III**). Moreover, the interaction of both leaf species and exposure history (i.e., different AH community structure and composition) results in a sex-specific change of gammarids' feeding strategies to different food sources (**Appendix III**). An unexpected result that raises questions on earlier procedures, where responses of only one sex or using undifferentiated sex were evaluated. In this context, sex-specific responses are not yet properly considered (**Appendix III**). Consequently, we hope future research will expand the replication using both sexes and looking into energy reserves to assess physiological responses of organism such as *Gammarus*. This demand for a more comprehensive assessment will hopefully develop the on potential bottom-up related effects in the wider food web.

This thesis provides a novel perspective on the effects of stressors in leaf-associated microbial communities and their potential wider effects. Therefore, our findings can be used as a basis for further and refined research to deepen the understansting on how leaf-associated communities respond to different chemical stressors and environmental factors. Moreover, as it shown in this thesis the role of defined traits of individual AH species is a key point to influence the function of these

microbial communities (i.e., leaf litter decomposition and increased nutritional quality for shredders). Thus, future studying should be designed to not only look into fungal individual traits but also include microbial colonisation dynamics, leaf species traits and sex-specific responses from shredder invertebrates, as suggested above.

This type of research is of up-most importance since worldwide increasing population and the consequent need for higher food production pressures agriculture expansion into pristine areas. Arable land-use is associated with the application of agrochemicals can affect local freshwater communities with consequences for the entire aquatic ecosystem. Under a climate change scenario, pests, such as fungi, have a higher chance to expand to higher latitudes. The latter together with agriculture land-use has the potential to change the leaf species composition and increases the frequency of pest control agents' application, increasing the potentially negative effects on freshwater communities. Further research has yet to be developed to deepen or understanding on how these stressors, factors and their interaction may jointly modify leaves' nutritional quality for shedders.

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7. APPENDIX

Appendix I and **III** represent the latest versions accepted by the respective journal. **Appendix II** is the latest version of the manuscript under review.

Appendix I

Leaf Species-Dependent Fungicide Effects on the Function and Abundance of Associated Microbial Communities.

Gonçalves, S., Post, R., Konschak, M., Zubrod, J., Feckler, A., & Bundschuh, M. Accepted in Bulletin of Environmental Contamination and Toxicology, 110(5), 1–7(2023). https://doi.org/10.1007/s00128-023-03728-2

Appendix II

Elevated Fungicide and Nutrient Concentrations Change Structure but not Function of Aquatic Microbial Communities.

Gonçalves, S., Feckler, A., Pollitt, A., Baschien, C., Michael, J., Schreiner, V. C., Zubrod, J. P., Bundschuh, M.

Accepted in Environmental Toxicology and Chemistry, 43(6), 1300–1311. https://doi.org/10.1002/etc.5863

Appendix III

Microbial community history and leaf species shape bottom-up effects in a freshwater shredding amphipod.

Gonçalves, S., Pollitt, A., Pietz, S., Feckler, A., & Bundschuh, M.

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7.1 APPENDIX I

Leaf species-dependent fungicide effects on the function and abundance of associated microbial communities

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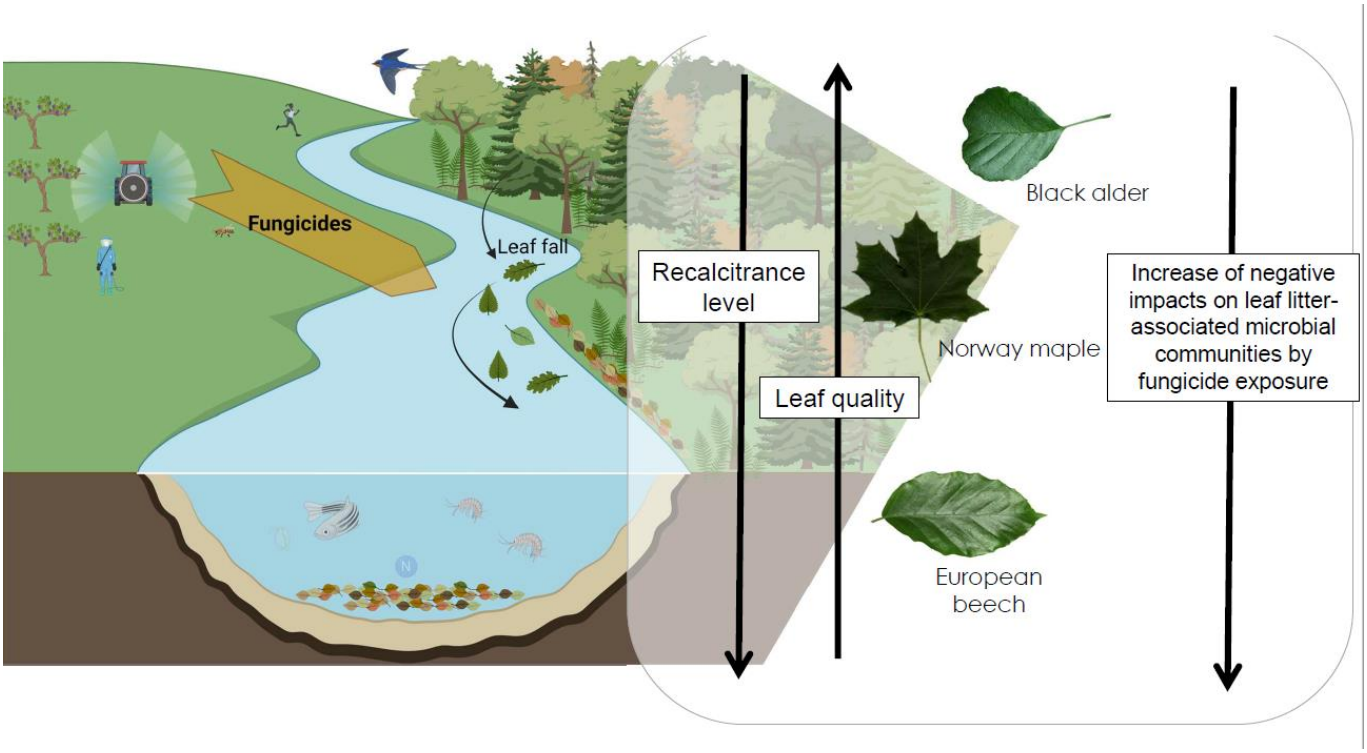
ABSTRACT

Microbially-mediated leaf litter decomposition is a critical ecosystem function in running waters within forested areas, which can be affected by fungicides. However, fungicide effects on leaf litter decomposition have been investigated almost exclusively with black alder leaves, a leaf species with traits favourable to consumers (i.e., low recalcitrance and high nutrient content). At the same time, little is known about fungicide effects on microbial colonisation and decomposition of other leaf species with less favourable traits. In this 21-day lasting study, we explore the effects of increasing fungicide sum concentrations (0 to 3000 µg/L) on microbial colonisation and decomposition of three leaf species (black alder, Norway maple and European beech) differing in terms of recalcitrance and nutrient content. Leaf litter decomposition rate, leaf-associated fungal biomass and bacterial density were quantified to observe potential effects at the functional level. Beech, as the species with the least favourable leaf traits, showed a substantially lower decomposition rate (50%) in absence of fungicides than alder and maple. In the presence of high fungicide concentrations (300-3000 µg/L), beech showed a concentration-related decrease not only in microbial leaf litter decomposition but also fungal biomass. This suggests that favourable traits of leaf litter (as for alder and maple) enable leaf-associated microorganisms to acquire leaf-bound energy more easily to withstand potential effects induced by fungicide exposure. Our results indicate the need to deepen our understanding on how leaf species' traits interact with the impact of chemical stressors on the leaf decomposition activity of microbial communities.

Keywords: recalcitrance level, leaf traits, aquatic fungi, fungicides

GRAPHICAL ABSTRACT



Created with BioRender.com

INTRODUCTION

Leaf litter decomposition is a key process in streams within forested catchments (Fisher and Likens 1973), which is inter alia driven by microbes such as bacteria and fungi, especially aquatic hyphomycetes (AH; Hieber and Gessner 2002). These microorganisms contribute directly to leaf litter decomposition, with their extracellular enzymes breaking down mono-, di- and polysaccharides (Evans and Hedger 2001). In this context, the efficiency of microorganisms to decompose leaf litter is assumed to be a function of microorganisms' species-specific characteristics (Baudy et al. 2021) as well as the chemical composition of leaf species (Melillo et al. 1982; Hladyz et al. 2009; Schindler, 2009). In fact, the levels of leaves' nutrients and structural (recalcitrant) components influence microbial colonization dynamics (Melillo et al. 1982; Webster and Benfield 1986; Gessner and Chauvet 1994).

In addition, anthropogenic chemicals are known to alter microbial colonization and decomposition of leaf litter. One group of chemicals that received increasing attention over the last decade is fungicides, which are designed to affect fungal pest species in agriculture (Zubrod et al. 2019). After their application, fungicides can reach surface water bodies, for example via runoff (Süß et al. 2014), where they interact with non-target organisms, such as microorganisms involved in leaf litter decomposition (Zubrod et al. 2011; Feckler et al. 2017). However, most studies addressing fungicide effects on leaf litter decomposition used black alder (*Alnus glutinosa* L. (Gaertn.)) as a model leaf species (e.g., Bundschuh et al. 2011; Fernández et al. 2015). While black alder may be considered representative of temperate riparian ecosystems (Bjelke et al. 2016), leaf litter of other tree species is also ecologically highly relevant (Gessner et al. 2010). As black alder leaf litter has a high nutrient content paired with a low share of recalcitrant substances, such as lignin (e.g., Melillo et al. 1982; Gulis 2001), it becomes the first to be colonized and decomposed by microorganisms. At the same time, the decomposition of other leaf species with less favourable traits happens slower, enabling the constant input of nutrients all year long (Gessner et al., 2010). Thus, the transferability of results obtained with black alder to other leaf litter species with deviating characteristics may be questioned.

In order to investigate the impact of different leaf species on the function of leaf-associated microbial communities under fungicide exposure, the present study made use of three leaf species with distinct characteristics: black alder (referred to as alder), which due to its characteristics has a slightly and substantially higher decomposition rate compared to Norway maple (*Acer platanoides* L.; referred to as maple) and European beech (*Fagus sylvatica* L.; referred to as beech; Gessner and Chauvet 1994; Abelho 2001). These leaf species were colonized by aquatic microorganisms while being exposed to increasing concentrations of a fungicide mixture over 21 days. Leaf litter decomposition rates were

quantified as a functional endpoint. Additionally, ergosterol content (as a proxy for fungal biomass) and bacterial density were measured to quantify microbial abundance. We expected (i) that alder and maple will be decomposed faster than beech in absence of fungicides, (ii) fungicides will negatively affect leaf-associated microorganisms' function, independent of the leaf species and (iii) the magnitude of fungicide effects on microbial leaf litter decomposition increases with increasing level of recalcitrance. This hypothesis is derived from the dynamic energy budget theory (Kooijman 2000) suggesting an elevated investment of energy to obtain nutrients from the leaves, leaving less for other processes including detoxification.

MATERIAL AND METHODS

Leaf material was collected in the vicinity of Landau, Germany: alder leaves were collected in autumn 2017 (49°11´N; 8°´5´O), while beech leaves and maple leaves were collected in autumn 2016 and 2015 (49°12´N; 8°´6´O), respectively. All leaves were stored at -20°C until use. To generate a near-natural inoculum of leaf-associated microorganisms, alder leaves were submerged in litterbags (mesh size: 0.5 mm; 10 leaves per bag) for 14 days in the Rodenbach, Germany (49°33´N, 8°´2´O). Subsequently, leaves were cleaned under tap water to remove adhering sediment and submerged for another 28 days in a stainless-steel channel filled with nutrient medium (Dang et al. 2005) being renewed every 7 days, under constant aeration and in darkness at 16 ± 1°C. Unconditioned alder leaves were added to generate an inoculum of various decomposition stages supposedly harbouring a higher fungal diversity (Gessner et al. 1993). This inoculum was subsequently used for the fungicide exposure assay.

For each leaf species, 150 unconditioned leaves were cut to strips (approximately 7.5 x 5 cm2). Leaf strips were leached for 24 h in nutrient medium to reduce potential impacts of leachates on microbially-driven leaf litter decomposition during the experiment (Gessner et al. 1999). Subsequently, leaf strips were dried at 60 °C for 24 h and weighted to the nearest 0.01 mg. Each replicate consisted of three dried and pre-weighed leaf strips, leading to a total of 50 replicates per leaf species to be evenly split among five fungicide treatments (n=10). The fungicide mixture used in the present study was composed of five fungicides covering a wide range of modes of action (Tab. S1). Fungicide test concentrations were chosen following earlier studies (e.g, Zubrod et al. 2015) using a spacing factor of ten: 0 (fungicide-free control), 3, 30, 300 and 3000 µg/L, with proper spiking being confirmed elsewhere (e.g., Zubrod et al., 2015b).

For the experiment, a fully-crossed 3x5-factorial test design was used. Each of the three leaf species was exposed to the five fungicide concentrations, including a fungicide-free control. Before test initiation, dried leaf strips were rehydrated for 24 h in nutrient medium before being introduced into mesh bags (mesh size: 0.5 mm). Mesh bags prevented the three leaf strips from sticking together and ensuring the accessibility of the leaf material for

microorganisms. Each replicate consisted of a 1-L glass beaker filled with 750 mL nutrient medium, 3 g microbial inoculum (wet weight; i.e., of pre-conditioned leaves), the three leaf strips as well as the fungicide mixture. Experiments were conducted at 16 ± 1°C under continuous aeration and in darkness. To avoid evaporation of nutrient medium, the beakers were covered with plastic foil, while the medium was renewed every seven days (including fungicide stocks). After 21 days, all leaf strips were removed from the test system and two leaf discs with a diameter of 16 mm were punched out of each leaf strip with a cork borer. One leaf disc from each leaf strip was used for leaf mass quantification and dried at 60°C for 24 h. The second leaf disc from each leaf strip was fixed in 2% formaldehyde solution (with 0.1% sodium pyrophosphate) and stored at 4°C for bacterial density analysis. The remaining material of the leaf strips was collected for leaf decomposition measurements as well as for ergosterol analysis and was stored at -20°C until further use. To quantify the leaf decomposition, the leaf discs for mass correction and the remaining leaf strips were freeze-dried for 24 h and weighed to the nearest 0.01 mg.

The leaf-associated ergosterol was quantified as a proxy for fungal biomass according to Gessner (2005). After extraction in alkaline methanol, ergosterol was purified by solid-phase extraction (Sep-Pak Vac RC tC18 500 mg sorbent, Waters) and quantified by high-performance liquid chromatography (1200 Series, Agilent Technologies). The bacterial density was quantified following (Buesing 2005). Briefly, bacterial cells were detached from the leaf discs using an ultrasonic probe (Sonopuls HD 2070 with TT 13 probe, both Bandelin, Germany) and filtered over aluminium oxide membrane filters (pore size 0.2 µm, Whatman). Filters were subsequently stained with SYBR Green II (Molecular Probes, Eugene, OR, USA). Twenty digital images were taken for each replicate under an epifluorescence microscope (Axio Scope.A1, Carl Zeiss Micro Imaging). Bacterial cells were counted using Axio Vision Rel 4.8 (Carl Zeiss Micro Imaging) and normalised to leaf dry mass.

The microbial leaf decomposition rate k (d^{-1}) was calculated following (Benfield 2007). Concentration-response models (including lognormal, log-logistic, Weibull, Cedergreen–Ritz–Streibig, and Michaelis–Menten models) were fitted separately for alder, beech and maple to assess the functional response to the five tested fungicide concentrations. The best-fitting models were selected based on visual judgment and Akaike's information criterion (all models and their respective parameters are reported in Tab. S4). The data on leaf decomposition, fungal biomass and bacterial density were checked for normal distribution and heteroscedasticity via Shapiro–Wilk and Levene's tests, respectively. Significant influences of the factors "fungicide treatment" and "leaf species" as well as their interaction were examined using rank-based two-way analyses of variance (ANOVA). For each leaf species, differences between control and individual fungicide treatments were checked with Wilcoxon rank sum tests followed by Bonferroni correction (Zar 2010). Moreover, we base our interpretation on

both statistical significance and effect sizes, considering the criticism of null hypothesis significance testing (i.e., the difference between treatments (Newman, 2009). R version 4.2.1 for Windows (R Core Team 2022) was used for the execution of the statistical tests and the creation of figures. The graphical abstract was created in BioRender.com.

RESULTS AND DISCUSSION

Leaf species significantly influenced the decomposition rate, fungal biomass and bacterial density (Fig. 1; Tab. 1 and 3; $p<0.001$). As hypothesised, beech leaves were decomposed slower than alder and maple in absence of fungicides. In general, alder leaves were decomposed fastest, followed by maple and beech (Fig.1). This observation is in accordance with former studies (e.g., Abelho, 2001) and is likely explained by a higher content of recalcitrant substances, such as lignin, in combination with low levels of nutrients in beech leaves (Melillo et al. 1982; Bastias et al. 2018). These leaf characteristics should restrict the colonisation of beech leaves by microbes, which in turn slows down decomposition. In contrast, leaf litter characterised by a lower recalcitrance and an elevated nutrient content (mainly nitrogen; Gulis, 2001), such as maple and alder, should also support fungal growth and consequently being more efficiently degraded (Artigas et al. 2004; Graça and Canhoto 2006).

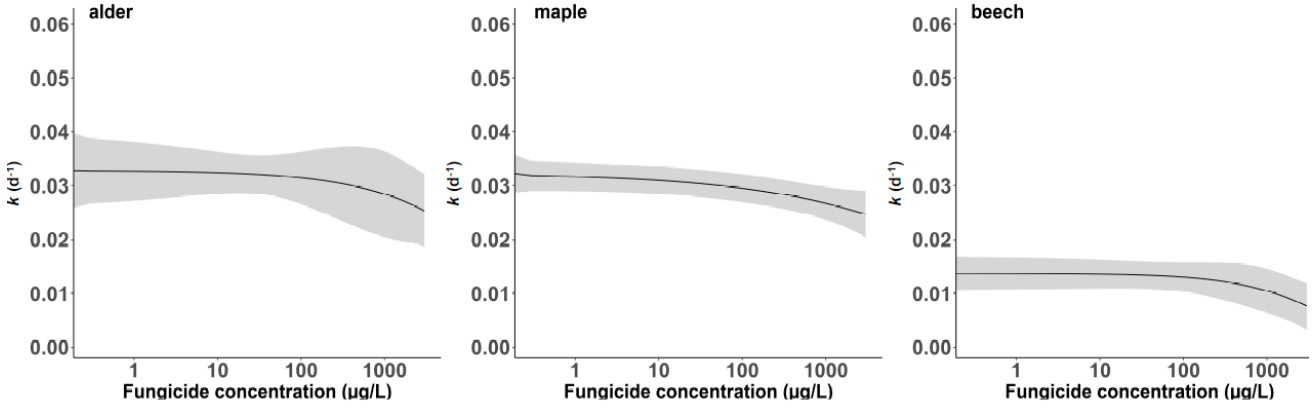


Figure 1. Concentration-response models (solid lines; shaded lines indicating corresponding 95% CIs; n = 10) for the leaf litter decomposition rate, k (d-1), as a function of the total fungicide concentration for the different leaf species alder, maple and beech.

In this study, alder was decomposed faster than maple and beech despite lower levels of alder-associated fungal biomass (Fig. 1; Tab. 2). Fungal biomass ignores the AH (aquatic hyphomycete) species composition and the potential replacement of less active fungal species by species with a higher decomposition efficiency (Baudy et al. 2021). Moreover, the alder-associated fungal biomass might have already peaked before the termination of the experiment (Baldy et al. 1995). This assumption is supported by Artigas et al. (2012), who

reported a peak in alder-associated ergosterol levels after 14 days under optimal conditions. Contrarily, for maple and beech, the maximum of ergosterol may not have been reached at test termination.

Table 1. Output for statistical analysis of the rank-based ANOVA. Degrees of freedom (Df); sum of squares (Sum Sq); mean squares (Mean Sq). P-values printed bold indicate statistical significance. Table taken from Appendix II.

Enpoint	Method	Source of variation	Df	Sum Sq	Mean Sq	F value	P-value
Leaf litter decomposition rate	ANOVA	**Leaf species**	2	0.0107	0.0054	66.394	**p < 0.001**
		Fungicide	4	0.0009	0.0002	2.824	**0.027**
		Leaf species x fungicide	8	0.0002	0.0001	0.387	0.926
		Residuals	135	0.0108	0.0001		
Fungal biomass (ergosterol)	ANOVA	**Leaf species**	2	396.2	198.1	21.118	**p < 0.001**
		Fungicide	4	2751.7	687.9	73.341	**p < 0.001**
		Leaf species x **fungicide**	8	290.5	36.3	3.872	**p < 0.001**
		Residuals	135	1266.3	9.4		
Bacterial density	ANOVA	**Leaf species**	2	1.25×10^{18}	6.26×10^{17}	31.205	**p < 0.001**
		Fungicide	4	2.10×10^{17}	5.25×10^{16}	2.618	**0.038**
		Leaf species x fungicide	8	1.37×10^{17}	1.71×10^{16}	0.855	0.557
		Residuals	130	2.61×10^{18}	2.01×10^{16}		

Fungicide exposure negatively impacted leaf litter decomposition, fungal biomass and partially bacteria density for all leaf species (Fig.1, Tab.1 and 3; $p<0.05$). Although the observed effect sizes were small (5-12%), likely due to the fungicide concentrations not being high enough to impact fungicide-tolerant AH species (Zubrod et al., 2019), leaf litter decomposition rates decreased with increasing fungicide concentrations independent of the leaf species (Fig. 1). The interaction term of the factor “leaf species” and “fungicide” was non-significant ($p>0.9$; Tab.1 and S3, Fig. S1), which points to a similar response pattern of the microbial communities in terms of leaf litter decomposition among leaf species with increasing fungicide concentrations. Nevertheless, the highest reductions in decomposition rates varied by a factor of two (12 vs 21 and 20% reduction for alder, maple, and beech, respectively, between control and 3000 µg/L; Tab. S2) pointing to relevant differences between leaf species. While the reductions between the second highest (i.e.,300) and highest (i.e., 3000 µg/L) treatment were also noteworthy (i.e., 14%, 7% and 34% for alder, maple and beech, respectively). These reductions of leaf decomposition support the negative impact of the

fungicide mixture, which tended to increase with less favourable leaf species traits (higher recalcitrance and decreasing nutrient levels) and was particularly pronounced for fungal biomass (Tab. 2). In contrast to fungal biomass, bacterial density differed slightly between maple and alder but was reduced for beech, independent of the fungicide concentrations. Hence, consistent pattern in bacteria density was not observed, supporting their minor contribution to leaf decomposition (Hieber and Gessner 2002).

Table 2. Bacterial density, as number of cells per mg leaf dry weight, and ergosterol concentration, as µg per mg of leaf dry weight, of different leaf species (alder, maple, and beech) ± 95% CIs., for the increasing fungicide concentrations. Table taken from Appendix II.

Leaf species	Fungicide concentration (µg/L)	Bacterial density (number of cells 10^8/mg leaf dw)		Ergosterol concentration (µg/mg leaf dw)	
	0	3.04	± 0.68	8.40	± 1.17
	3	3.33	± 0.44	6.55	± 1.07
alder	30	2.08	± 0.21	6.90	± 1.10
	300	2.48	± 0.40	4.86	± 0.92
	3000	2.40	± 0.29	0.56	± 0.15
	0	3.49	± 0.27	14.11	± 0.80
	3	4.60	± 0.79	14.79	± 1.00
maple	30	3.90	± 0.64	11.03	± 0.99
	300	2.56	± 0.19	5.90	± 0.82
	3000	3.52	± 0.28	0.82	± 0.06
	0	1.33	± 0.10	12.70	± 0.75
	3	1.53	± 0.24	11.82	± 1.20
beech	30	1.67	± 0.19	11.54	± 1.03
	300	0.88	± 0.10	3.87	± 0.43
	3000	1.51	± 0.08	0.14	± 0.04

For the tested fungicide concentrations, no significant changes in decomposition rates were found for alder in comparison to the control. In a previous study (Zubrod et al. 2015) with the same fungicide mixture at comparable concentrations, however, significant changes in the leaf decomposition rate were detected for alder, which might be related to a substantially higher statistical power due to higher replication (n=49) relative to the present study (n=10). Nonetheless, the effect size observed for alder at the highest fungicide concentration (i.e., 3000 µg/L) is in accordance with Zubrod et al. (2015). For the other leaf species, the decomposition rate was affected similarly between maple and beech, with effect size being twice as high when compared to alder. Maple and beech showed a non-significant reduction in the leaf decomposition rate of up to ~20% at the two highest fungicide concentrations (300-3000 µg/L). Changes in fungal biomass support this pattern (see also Zubrod et al. 2015a), with a lower reduction of the ergosterol concentration on alder relative to beech or maple

among fungicide treatments (Tab. 2). Moreover, fungal biomass was the only evaluated endpoint to show an interaction between leaf species and fungicide exposure, suggesting a non-additive effect of both variables. Based on our within species data, the latter findings suggest that traits of alder leaves (high nutrient levels and low recalcitrance) enable leaf-associated microorganisms to acquire leaf-bound energy more easily to withstand potential effects induced by fungicide exposure (Solé et al. 2012). This interpretation has not been supported by statistical significance (Tab. 1), however it is backed by fungal biomass data being more reduced under fungicide exposure on the most recalcitrant and least nutrient-rich leaf species (namely beech) – an observation made by Artigas et al. (2012). In their study, the presence of 30 µg tebuconazole/L induced a 60% higher reduction in fungal biomass associated with more recalcitrant black poplar (*Populus nigra* L.) relative to alder. The discrepancies in fungicide effects between maple and alder, which both should be comparably well decomposable, might be related to maple having a relatively smooth surface on both leaf sides making colonisation and penetration by fungi more challenging (Kearns and Bärlocher 2008). Consequently, fungal propagules are exposed to fungicides for a longer duration. On alder, however, the fungal propagules can quickly attach and grow into the leaf (Kearns and Bärlocher, 2008), which may provide protection and reduced fungicide exposure. Moreover, some fungicides only act on the propagules of fungi and not on growing mycelium (Escudero-Leyva et al. 2022). While this aspect seems of little relevance in absence or at low levels of fungicides, the combination of leaf surface traits with fungicide stress may have contributed to the more pronounced fungicide effect at higher concentrations in beech and maple leaves. Similarly, bacterial density was not substantially affected by fungicide exposure (Tab. S3), suggesting again a minor relevance of leaf recalcitrance and nutrient content for bacterial colonisation (Feckler et al., 2017).

CONCLUSION

Overall, this study shows that higher recalcitrance and lower nutrient levels in leaf litter potentially may lead to increased fungicide effects during its decomposition. This seems particularly relevant in the light of alder replacement in riparian zones over the last decades across Europe due to different causes, such as habitat exploitation and pathogen infections (Brasier et al. 1995, 1999, 2004; Graça and Canhoto 2006; Richardson et al. 2007; Husson et al. 2015). Therefore, changes in tree species composition along riverbanks are expected (Bjelke et al. 2016) further diversifying the leaf litter and its susceptibility to be decomposed. Thus, understanding the leaf litter decomposition activity of local microbial communities is essential to expand our research on how leaf litter traits interact with the impact of chemical stressors.

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Supplementary information for the paper:

Leaf species-dependent fungicide effects on the function and abundance of associated microbial communities

Table S1. Information on the fungicide mixture components, their product names, manufacturers, active ingredient concentrations, nominal concentrations, and mode of action.

Substance	Product name	Manufacturer	Active ingredient concentration	Nominal concentrations (µg/L)	Mode of action according to the Fungicide Resistance Action Committee (2017)
Azoxystrobin	Ortiva	Syngenta Agro	250 g/L	0; 0,5; 5; 50; 500	Inhibition of mitochondrial respiration
Carbendazim	Derosol	Bayer Crop Science	600 g/kg	0; 0,5; 5; 50; 500	Inhibition of mitosis and cell division
Cyprodinil	Chorus	Syngenta Agro	500 g/kg	0; 0,5; 5; 50; 500	Inhibition of amino acid and protein synthesis
Quinoxyfen	Fortess 250	Dow Agro Science	250 g/L	0; 1; 10; 100; 1000	Perturbation of signal transduction
Tebuconazol	Folicur	Bayer Crop Science	250 g/L	0; 0,5; 5; 50; 500	Inhibition of sterol biosynthesis

Table S2. Leaf litter decomposition rate, k, per day, of increasing total fungicide concentrations for the different leaf species alder, maple, and beech.

Leaf species	Fungicide concentration (µg/L)	Leaf litter decomposition rate ± sd		
Alder	0	0.034	±	0.013
	3	0.032	±	0.014
	30	0.029	±	0.011
	300	0.030	±	0.005
	3000	0.026	±	0.011
Maple	0	0.031	±	0.005
	3	0.034	±	0.006
	30	0.031	±	0.010
	300	0.026	±	0.007
	3000	0.025	±	0.008
Beech	0	0.013	±	0.006
	3	0.012	±	0.005
	30	0.015	±	0.007
	300	0.012	±	0.012
	3000	0.008	±	0.007

Table S3. Statistical output of pairwise comparisons between the individual fungicide concentrations using Wilcoxon rank sum tests with subsequent Bonferroni correction. P-values printed bold indicate statistical significance.

Endpoint	Comparision	alder	beech	maple
Leaf litter decomposition rate	0-3	1	1	1
	0-30	1	1	1
	0-300	1	1	1
	0-3000	1	0.4	0.63
	3-30	1	1	1
	3-300	1	1	0.185
	3-3000	1	1	**0.052**
	30-300	1	1	1
	30-3000	1	0.45	1
	300-3000	1	1	1
Fungal biomass (ergosterol)	0-3	1	1	1
	0-30	1	1	0.23231
	0-300	0.3428	**0.0001**	**0.00011**
	0-3000	**0.0018**	**0.00163**	**0.00011**
	3-30	1	1	0.28806
	3-300	1	**0.01505**	**0.00022**
	3-3000	**0.0027**	**0.00163**	**0.00011**
	30-300	1	**0.00022**	0.0105
	30-3000	**0.0061**	**0.00163**	**0.00011**
	300-3000	**0.0044**	**0.00163**	**0.00487**
Bacterial density	0-3	1	1	1
	0-30	1	1	1
	0-300	1	0.147	0.29
	0-3000	1	1	1
	3-30	0.74	1	1
	3-300	1	0.63	0.75
	3-3000	1	1	1
	30-300	1	0.068	0.35
	30-3000	1	1	1
	300-3000	1	**0.015**	0.19

Table S4. Fitted models and their respective parameterization separated by leaf species.

Leaf species	Model	Lower limit	Parameters	
alder	Weibull type (3 parameters)	0	b:	0.532
			c:	0.033
			e:	38965.000
maple	Log-logistic (log(ED50) as parameter)	0	b:	0.351
			c:	0.032
			e:	11.423
beech	Weibull type (3 parameters)	0	b:	0.712
			c:	0.014
			e:	6372.800

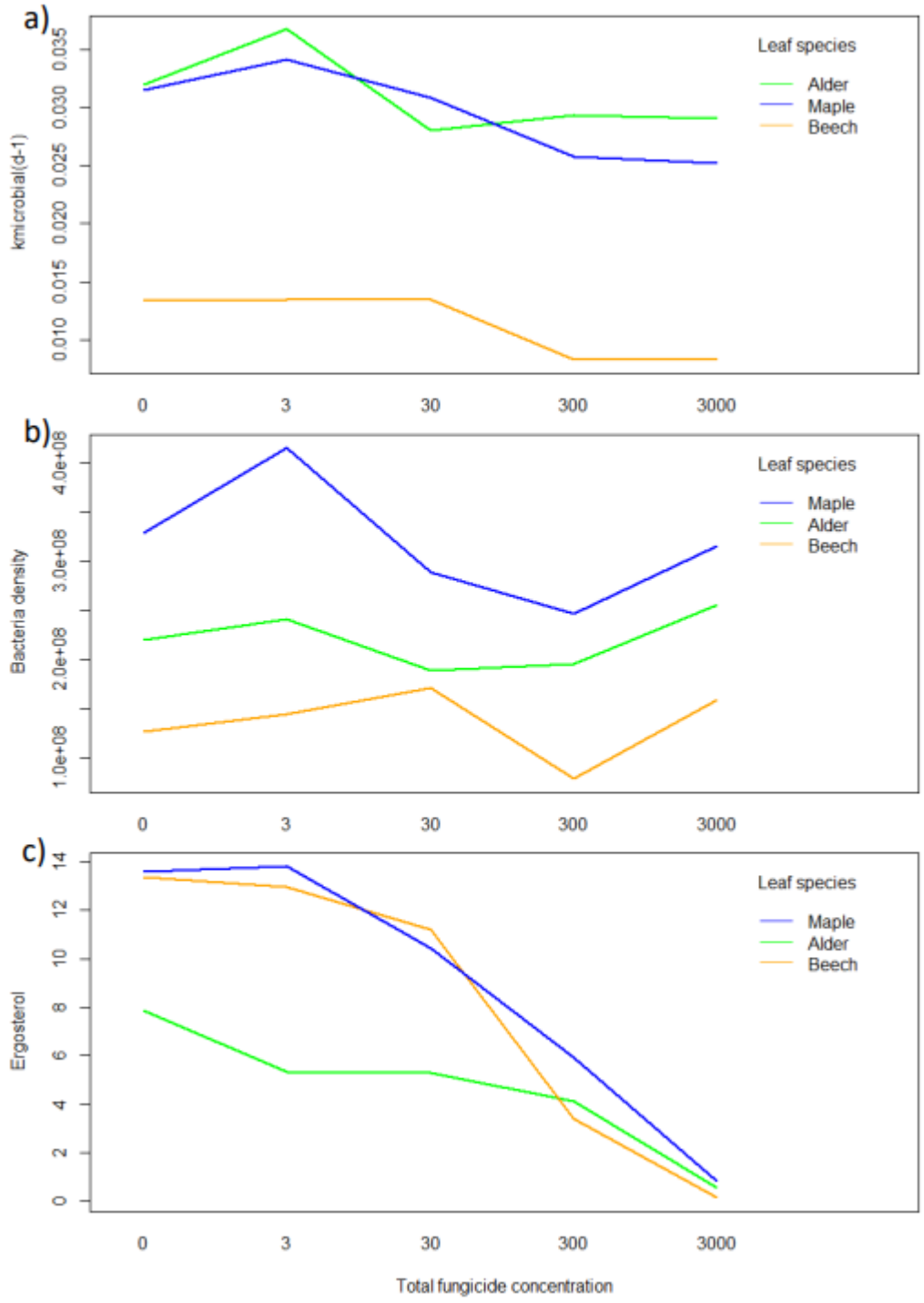

Figure S1. Interactions plots between factors “Fungicide” and “Leaf species” for a) Leaf decomposition rate; b) Bacterial density; and c) Ergosterol (Fungal biomass estimate). Lines in green, blue and orange indicate different leaf species, Alder, Maple and Beech, respectively. If the two lines on the interaction plot are parallel, then there is no interaction effect. If the lines intersect, then there is likely an interaction effect.

7.2 APPENDIX II

Elevated Fungicide and Nutrient Concentrations Change Structure but not Function of Aquatic Microbial Communities.

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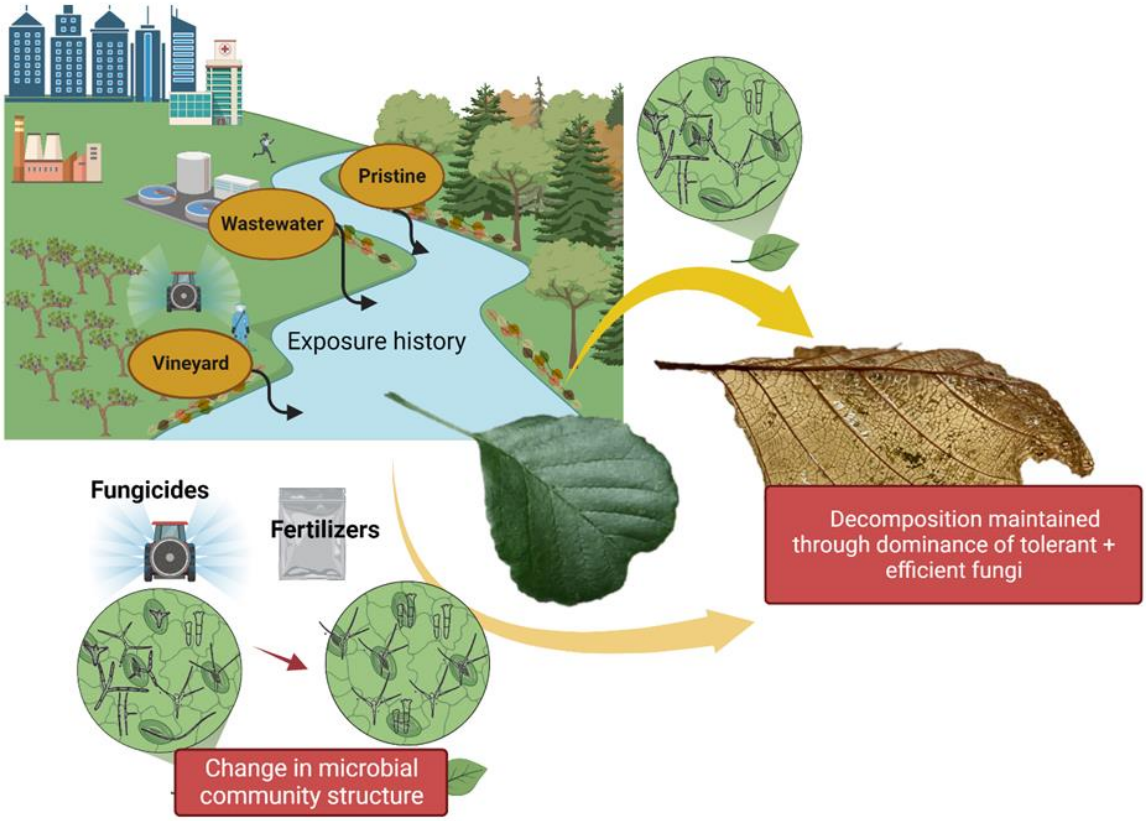
ABSTRACT

Leaf decomposition is a key process in stream ecosystems within forested catchments driven by microbial communities, particularly fungi and bacteria. These microorganisms make nutrients and energy bound in leaves available for wider parts of the food web. Leaf-associated microorganisms are subjected to anthropogenic pressures, such as the increased exposure to nutrients and fungicides associated with land-use change. In this study, we assessed the sensitivity of leaf-associated microbial communities with differing exposure histories, namely from pristine (P) streams, and streams impacted by wastewater (W) and agricultural run-off (vineyards; V). In the laboratory, microbial communities were exposed to increasing nutrient (NO_3-N: 0.2-18.0 mg/L, PO_4-P: 0.02-1.8 mg/L) and fungicide concentrations (sum concentration 0-300 µg/L) in a fully crossed 3x4x4-factorial design over 21 days. Leaf decomposition and exoenzyme activity were measured as functional endpoints, while fungal community composition and microbial abundance served as structural variables. Overall, the results showed that leaf decomposition did not differ between fungicide treatments or exposure histories. Nonetheless, substantial changes of the fungal community composition were observed when exposed to environmentally relevant fungicide concentrations. The observed changes in the fungal community composition support the principle of species dominance, with highly efficient decomposers maintaining leaf decomposition; potentially at the expense of other functions provided by fungi.

KEYWORDS: leaf decomposition, community structure, land-use, exposure history

GRAPHICAL ABSTRACT



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INTRODUCTION

Leaf litter of terrestrial origin represents a significant energy source for aquatic ecosystems, such as rivers and streams within forested catchments (Fisher & Likens, 1973). The energy stored in leaf litter is made available to wider parts of the food web through leaf decomposition, which represents a key ecosystem process (Minshall, 1967; Nelson & Scott, 1962). For this process, bacteria and fungi are considered central (Dighton & White, 1983; Webster, 2007). Through their extracellular enzymatic capability, these microorganisms convert recalcitrant oligo- and polysaccharides into assimilable mono- and disaccharides, ultimately fuelling a wider part of the food web (Boulton & Boon, 1991; Hieber & Gessner, 2002).

Leaf decomposition in rivers and streams is, however, influenced by the catchments' land-use and associated stressors. For example, the influx of nutrients and pesticides into surface waters, which have been linked to agricultural land-use (Tilman et al., 2001), affects leaf-associated microbial communities. While nutrients generally stimulate microbial activity up to a certain concentration (Ferreira et al., 2015), fungicides are mainly associated with a reduction in leaf decomposition (e.g., Fernández et al., 2015; Zubrod et al., 2015). Moreover, the microbial communities' functional response to fungicides and nutrients is influenced by the communities' exposure histories (Feckler et al., 2018; Gardeström et al., 2016). In fact, the functional tolerance of leaf-associated microbial communities, measured through their leaf decomposition rate, towards fungicides was observed to be higher when sampled from streams impacted by agriculture (i.e., with exposure history) compared to near-natural streams (i.e., without exposure history; Feckler et al., 2018). This observation suggests that previous exposure to fungicides acts as a filter selecting for tolerant (and partly more efficient in terms of leaf decomposition) species, with the fungal group of aquatic hyphomycetes (AH) being considered as the its major driver (Gessner et al., 2007).

An earlier study (Feckler et al., 2018) acknowledged that the general applicability of the findings requires an expansion of true replicates (i.e., microbial communities with and without an "exposure history"). Our study expands the dataset by sampling from streams associated with different land uses and increasing the number of replicates at each site, as a more robust basis of comparison for earlier findings. Leaf-associated microbial communities were sampled from pristine (P) streams, and streams impacted by wastewater (W) as well as run-off from the locally dominating crop, namely vineyards (V), each independently replicated three times (i.e., nine sites in total). It was expected that leaf-associated microbial communities from V-impacted stream sections structurally and functionally adapted to moderate nutrient and high fungicide exposure, representing the major chemical stressors used in such catchments (Tilman et al., 2001; Zubrod et al., 2019; Fernández eta al., 2015). Microbial communities impacted by W are

expected to be adapted to relatively high nutrient concentrations, while being exposed to a broad range of organic micropollutants including fungicides. Within the same sampling region, leaf-associated microbial communities sampled from P-streams were included to establish a baseline for the microbial communities' responses to fungicides and nutrients (sampling region as in Fernández et al., 2015).

In the laboratory, these microbial communities were exposed to environmentally relevant but increasing nutrient and fungicide concentrations, involving a fully crossed 3x4x4-factorial design over 21 days. Besides microbially-mediated leaf decomposition, we analysed the communities' exoezyme activities as well as fungal and bacterial abundances approximated by real-time polymerase chain reaction (qPCR), and fungal community compositions through next generation sequencing (NGS). We hypothesized that (i) microbially-mediated leaf decomposition will be reduced with increasing fungicide levels, while the effects will be more pronounced for microbial communties from P-streams than for W- and V-streams (see Feckler et al., 2018). This leaf decomposition pattern (ii) should be reflected in a higher activity of enzymes degrading recalcitrant carbon in W- and V- compared to P-communities, due to the colonisation of leaves by more tolerant microbial communities with higher enzymatic capabitily (e.g., Baudy et al., 2021). Moreover, (iii) increasing nutrient levels should buffer the negative fungicide effects through the provisioning of additional and easily assimilable energy compared to treatments with lower nutrients (e.g., Ferreira et al., 2015 but see Fernández et al., 2016). Finally, (iv) changes in leaf decomposition in response to elevated nutrient and fungicide exposure are linked to shifts in the community structure (bacterial, fungal abundances and fungal community composition) favouring more tolerant and more efficient AH species. In this context, community changes were expected to be more prominent in P- than for W- and V-communities, with the latter being already shaped through exposures.

MATERIAL AND METHODS

General experimental design.

The exposure histories of the leaf-associated microbial communities were defined by the land-uses upstream of the sampling sites (Fig. 1). Factors as different soil properties, light availability, photosynthetic differences of the independent sites are might change the properties of the leaves and leaf-associated microbial communities, they were in the present study consider as naturally part of the factor exposure history. The communities were sampled from pristine streams with forest-dominated catchments (P; sites P1, P2 and P3 as replicates), as well as from streams impacted by either wastewater discharge (W; sites W1, W2 and W3 as replicates) or vineyard run-off (V; sites V1 and V2 as replicates; severe draughts during autumn 2019 did not allow to assess V3; see Table S1). We performed three independent

semi-static bioassays in April/May (sites P1, W1 and V1), July/August (sites P2, W2 and V2) and September/October (sites P3, W3 and V3) in 2019. Each of the bioassays, was planned to include one community per exposure type (i.e., P-, W- and V-community), following a 3x4x4-factorial design with a duration of 21 days (Fig. 1, 2 & 3; Table S1). Such a sequential procedure was employed as the number of experimental units (i.e., 720) for the entire experiment would not have been manageable in parallel.

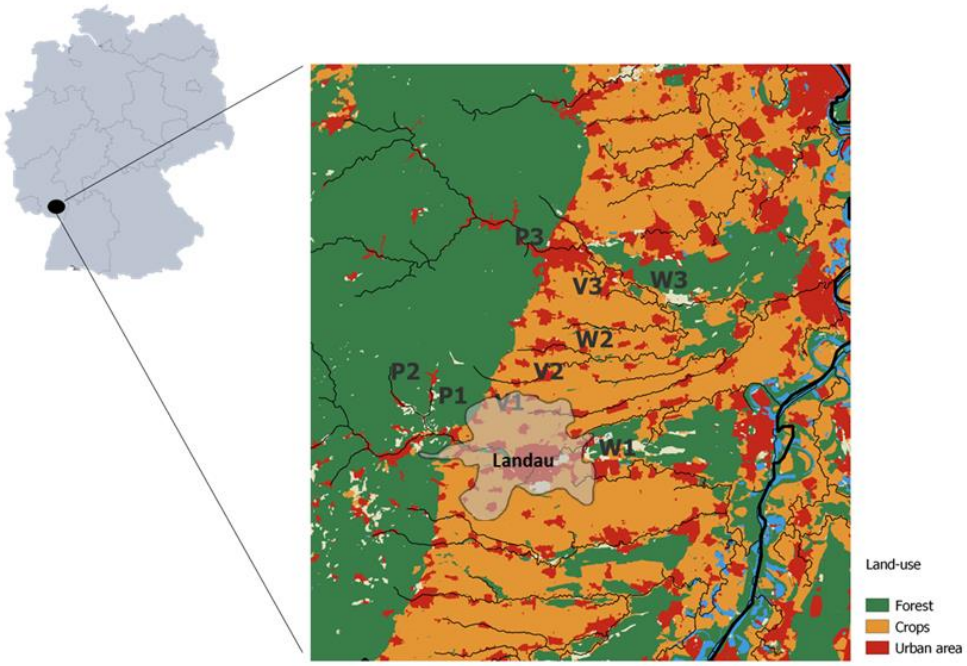

Figure 1. Map of the major land-use for the sampling region. Green, orange, and red represent forest, crops and urban area, respectively. Dark lines represent major stream segments. Letters represent different land-use categories upstream of the sampling sites, i.e., pristine – P (1-3), wastewater treatment effluent - W (1-3), and vineyard - V (1-3) and their catchments based on Sentinel-2 10 m land-use map (Karra et al., 2021).

During each of these bioassays, pre-stored black alder (*Alnus glutinosa* (L.) Gaertn.) leaves were deployed in the respective stream and let to colonise by microorganisms (see section Preparation of microbial inocula and leaf material).. Later in the laboratory, microorganisms were exposed to four increasing concentrations of a fungicide mixture (0-300 µg/L; Table S2; see section Chemicals) as well as four nutrients concentrations. The nutrient and fungicide mixture concentrations were selected based on previous studies (Feckler et al., 2018; Zubrod et al., 2015). The nutrient medium composition largely followed Dang et al. (2005), but with adjusted NO_3-N (0.2, 2.0, 10.0 and 18.0 mg/L) and PO_4-P (0.02, 0.2, 1.0 and 1.8 mg/L) concentrations at a fixed ratio of 10:1 (Fig. 3) to mimic a natural nutrient gradient in streams (Feckler et al., 2018). In the following, these nutrient concentrations are referred to as

very low, low, moderate and high. The fully crossed design resulted in a total of 48 treatments, each replicated five times.

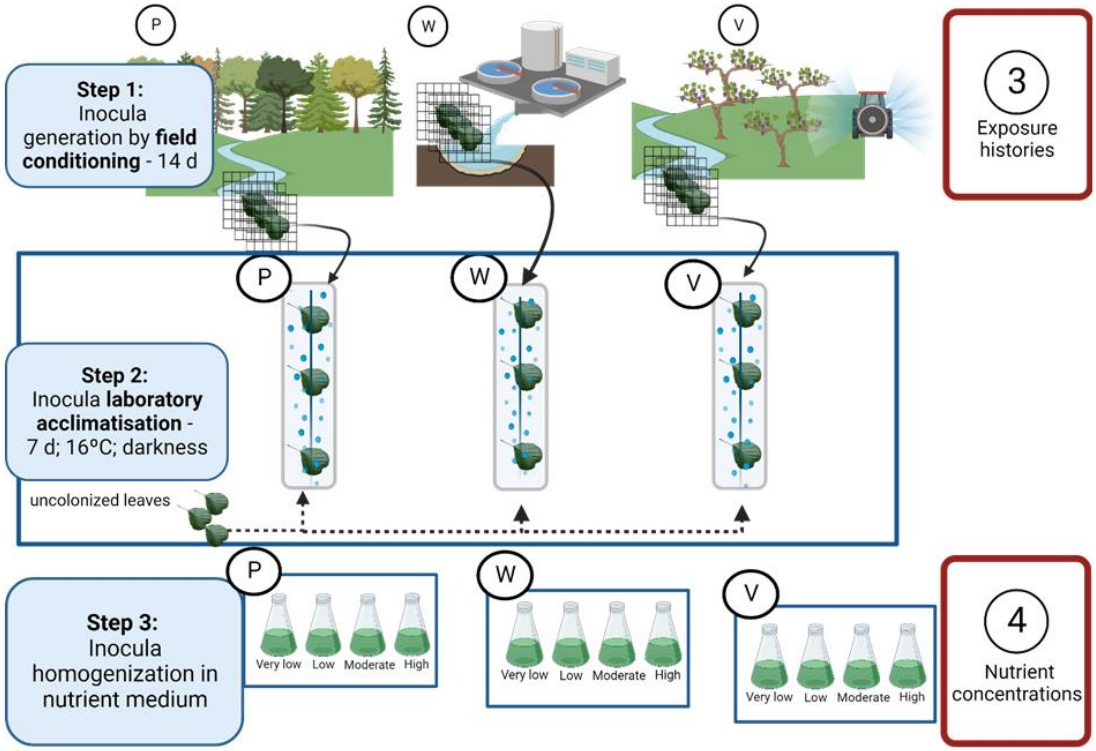

Figure 2. Schematic overview of the inocula preparation. Step 1: Generating inocula from pristine (P) streams, or streams impacted by wastewater discharge (W) and vineyard run-off (V) by deploying alder leaves in the field for 14d; Step 2: Inocula acclimatisation to laboratory conditions; leaves from each sampling site and uncolonized leaves are further microbially colonized for 7 d; Step 3: Inocula (leaves) homogenisation in nutrient media per exposure history and respective Created with BioRender.com

Preparation of microbial inocula and leaf material.

The microbial inocula were obtained from streams near Landau, Germany (Table S1; Fig. 1), by submerging black alder leaves in litterbags (10 leaves with different sizes per bag; 15 x 15 cm; mesh size = 1 mm; n = 50) at each sampling site for 14 days (Fig. 2). Leaf material originated from trees within the same region sampled before abscission during autumn 2017 and 2018 was visually inspected for damages and infections (excluded) and divided per size (stored at -20 °C until use). Freezing may cause minor changes in leaf decomposition (Bärlocher 1992; Boyero et al., 2016), only relevant when extrapolating to field conditions. After field colonization, the leaf material was transported to the lab in stream water. In the laboratory, leaves were carefully cleaned from invertebrates and sediment particles under running tap water. This previous step can potentially change the microbial assemblages; however, it is the same for all replicates and necessary, as the impact of invertebrates' feeding could confound our final results heavily. The inoculum from each sampling site was subsequently placed in an individual stainless-steel container (120 × 30 × 20 cm; volume 50 L) filled with 25 L of constantly

aerated stream water from the respective sampling site at 16 ± 1 ºC in darkness for seven days. In addition, another 500 uncolonized black alder leaves were added to increase habitat diversity enhancing the chances of maintaining a diverse microbial community, driven by two stages of leaf decomposition (Gessner et al., 1993).

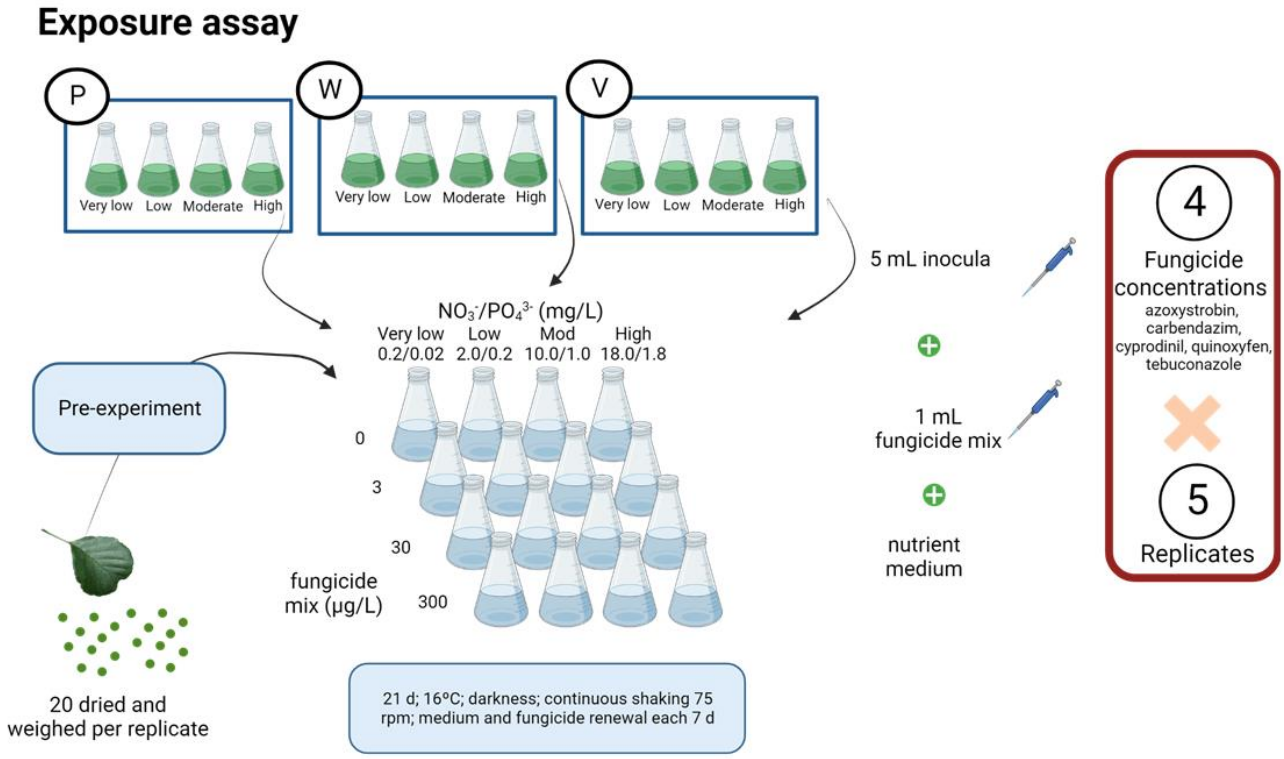

Figure 3. Exposure assay – the inocula prepared were used to microbially colonize leaf discs in Erlenmeyers flasks, while being exposed to increasing concentrations of nutrients and fungicides over 21 d, with media and fungicides being renewed every 7 d. Created with BioRender.com

Chemicals.

The fungicide mixture consisted of five active ingredients, namely azoxystrobin, carbendazim, cyprodinil, quinoxyfen, and tebuconazole, contained in pesticide formulations commonly applied in the region (Landesamt für Umwelt, 2016). The modes of toxic action, active ingredients and respective manufactures of the fungicide formulations are presented in Table S2. Total nominal concentrations used were 0 (control), 3 & 30 (environmental relevant concentrations), and 300 µg/L (high contamination). To confirm nominal concentrations of the individual fungicides, samples were taken from the test Erlenmeyer's approximately 2 h after test initiation as well as just before the weekly medium exchange (see section "Exposure assay") and analysed using liquid chromatography– high resolution mass spectrometry (Thermo Fisher Scientific, Dreieich, Germany) following published protocols (as in Fernández

et al., 2014; SI A.2.1). Although measured sum concentrations deviated partly by up to 30% from the nominal levels (Table S3), mainly due to insufficient quantification limits (3 µg/L) or potential fungicide attachment to leaf material, the spacing factor between tested concentrations was reached justifying the use of nominal concentrations in the following.

Exposure assay.

Prior to test initiation, leaf discs (Ø 20 mm) were cut from frozen and uncolonised leaves, pooled in groups of 20, dried at 60 ºC for 24 h, and weighed to the nearest 0.01 mg. Forty-eight hours before the initiation of each bioassay, dried and pre-weighted leaf discs were leached in autoclaved nutrient medium with treatment-matched nutrient concentrations. This is an important step to reduce potentially confounding impacts of leachates released from fresh leaves. Five additional replicates per nutrient concentration were included, which were used to correct for additional leaching-induced and physical leaf mass loss. Furthermore, 9.9 g wet weight leaf material from the stainless-steel containers (see above) were transferred to 150 mL of nutrient medium with treatment-matched nutrient levels and homogenised on ice using an Ultra-Turrax® T25 (IKA®-Werke, Staufen, Germany) to generate microbial inocula suspensions. Subsequently, 5 mL of these suspensions, 20 pre-weighted and leached leaf discs, and 1 mL of fungicide stock solution were transferred into sterilized 150 mL Erlenmeyer flasks, and autoclaved nutrient medium was added to reach a final volume of 50 mL. Erlenmeyer flasks were closed with sterile culture cellucotton plugs allowing air exchange, kept at 16 ± 1 ºC in darkness under continuous orbitally shaking at 75 rpm, while the nutrient medium together with the fungicide mixture was renewed every seven days. After 21 days, the bioassay was terminated and leaf discs were recovered. From the 20 leaf discs, two random leaf discs were analysed of the leaf-associated microbial communities and one leaf disc was used to quantify exoenzyme activities. For these purposes, leaf discs were lyophilized and weighed to the nearest 0.01 mg. The dry weight of the remaining 17 discs (dried at 60 °C for 24 h and weighed to the nearest 0.01 mg) was used to estimate the microbially-mediated decomposition rates (see data analysis section for details; Benfield, 2007).

Exoenzyme activity.

Hydrolase and oxidase activities were quantified using the method described by DeForest (2009) but modified for its use to analyse leaf litter (see Baudy et al., 2021). Detailed information is provided in the Supplementary Information (SI) A.2.2. Enzymatic activities were expressed as µmoL of degraded substrate/mg leaf dry weight/hour (DeForest, 2009). Subsequently, the data was used to calculate the recalcitrance ratio of the leaf material as normalised oxidases per total hydrolases activities (Table S4). The higher the ratio of oxidase to hydrolase activities, the greater is the relative investment for degradation of recalcitrant carbon (Romero-Olivares et al., 2017).

Characterisation of leaf-associated microbial communities.

Fungal and bacterial abundances. The FastDNA® Spin Kit for Soil in combination with the FastPrep™-24 5G Instrument (MP Biomedicals, Germany) was used to extract DNA from leaf material. In addition, we processed empty extraction tubes as negative controls in each extraction run. The amounts of fungal and bacterial operon copies were quantified as proxies for overall leaf-associated fungal and bacterial abundances, respectively, via SYBR® Green reactions (Manerkar et al., 2008). qPCR solutions with a total of 10 µL consisted of 2.8 µL of DNAse free water, 0.1 µL of forward primer, 0.1 µL of reverse primer (both at 10 µmoL/µL, from biomers.net GmbH, Ulm, Germany, see more details in Table S5), 2 µL of 50-fold diluted DNA extract, and 5 µL of PowerUp™ SYBR® Green Master Mix (Applied Biosystems Massachusetts, USA). PCR cycling conditions consisted of initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 15 s, and extension at 72 °C for 60s. At the end of each run, a melting curve analysis was performed to ensure the specificity of the assays. qPCR reactions were performed on a Mastercycler® ep gradient S (Eppendorf, Hamburg, Germany) using 0.2-mL 8-tube strips covered with clear optical 8-cap strips (Sarstedt AG & Co. KG, Nümbrecht, Germany). Results were dry weight normalized to the respective leaf discs. Further details on the assays are provided in the Supplementary Information (Table S5).

Fungal community composition. The DNA extracts (see above) were used to perform NGS according to the protocol in Carl et al. (2022). For each of the studied communities (P1-3, V 1-2 and W1-3), three levels of fungicides (0, 30 and 300 µg/L) and nutrients (very low, low and high) were evaluated, omitting the low and medium concentrations, respectively. This narrowed focus is motivated by the expected effects at higher fungicide concentration and the fact that these nutrient concentrations reflect the range reported for the sampling sites (Table S1) or excess of nutrients compared to sampling sites (high concentration).

Preparation of leaf samples for sequencing on the Illumina MiSeq are described in Carl et al. (2022), with detailed information being provided in SI A.2.3. Amplicon libraries of the fungal ITS2 rDNA gene were generated using a mix of five forward primers ('ITS3tagmix') and one reverse primer ('ITS4ngs';Tedersoo et al., 2014, 2015). PCR products were pooled for each sample to account for the technical bias of PCR reactions (Lindahl et al., 2013). For metabarcoding, barcodes, sequencing adaptors, and indices were ligated to the products of the first PCR. The resulting ITS2 library was sequenced on the Illumina MiSeq System using the chemistry of a 600-cycle MiSeq Reagent Kit v3 (Illumina, San Diego, USA). Indices were demultiplexed, followed by barcode demultiplexing using an inhouse script of Leibniz Institute DSMZ (https://github.com/boykebunk/amplicon). Sequences were processed with PIPITS (Version 2.4, Gweon et al., 2015, https://github.com/hsgweon/pipits/releases), Taxonomic

assignment was performed using the trained datasets of the Ribosomal Database Project (RDP) classifier (UNITE DB version February 02, 2019). Of this, PIPITS created an OTU (operational taxonomic unit) table for every sample, which was assigned according to the 'Species Hypothesis' (SH) of the UNITE database (Nilsson et al., 2019). Classification of OTUs was curated as described in Carl et al. (2022). In brief, (i) classification assigned to OTUs was re-blasted against NCBI reference databases (nucleotide collection of GenBank BLAST®; megablast within 'blastn' web application; https://blast.ncbi.nlm.nih.gov/Blast.cgi), (ii) corrected, if necessary, as detailed in Carl et al. (2022), and (iii) OTUs assigned to the same species hypothesis were merged to one taxon to lessen the marker bias of the ITS region, OTUs leading to the same species curation were merged per sample. The criteria used for the curation of each OTU were: (i) significant similarity to any BLAST-hit of a fungal taxon (≥95%), (ii) reasonable coverage of sequence (≥95%), (iii) highest e-value (ratio between coverage and similarity of the sequence), and (iv) reliably published sequence (reference database, isolate voucher, publication yes/ no) fungal ITS rDNA region (Heeger et al. 2018; Table S7). Within the whole dataset, 178 taxa passed our quality criteria. From these 178 taxa, those appearing only once were excluded from further analysis to reduce random noise, while this procedure did not influence the overall outcome of our analyses. The remaining 93 taxa were used to characterize the fungal community in each treatment (Table S6; S7).

Data analysis.

The variables "exposure history" and "season" (time of the sampling) were highly correlated (multicollinearity); thus, "season" was excluded from further analysis as this study was design to focus on "exposure history". Data obtained from microbial inocula collected from sampling sites with common land-use were used as replicates for data analysis. This pooling approach allowed us to generalize the findings and draw more robust conclusions about the microbial communities from P-, W- and V-streams and their responses to the experimental conditions. Microbially-mediated leaf decomposition rates, expressed as $k_{microbial}$ (d^{-1}), were calculated according to Benfield (2007):

$$k\mathit{microbial} = \frac{-\ln(dwf/(dwi * l))}{t}$$

where *dwf* and *dwi* refer to the final and the initial dry weights of leaf discs, *l* is a dimensionless empirical factor used to correct for the leaf mass loss due to leaching (which is dependent of the treatments and in this study ranged between 0.74-0.81), and *t* is the decomposition time (21 d). Subsequently, we fitted dose-response models ("drm"-command) on the leaf decomposition rates of each exposure history and nutrient level against fungicide concentrations. The best fitting models (always lower limit at 0) were chosen based on visual judgment and Akaike's information criterion (Table S8, for detailed information).

Shapiro–Wilk tests and Levene's tests were used to test for normality of residuals and homoscedasticity of univariate data (all data except fungal community composition). If the assumptions for parametric testing were met (only for enzyme activity), analyses were run on the original data by applying three-factor analyses of variance (ANOVA) with the independent variables, exposure history ("history"), fungicide exposure ("fungicide"), and nutrient concentration ("nutrient"), followed by post-hoc comparisons for main effects with Bonferroni p-value adjustment. Since the assumptions for parametric testing were violated for microbially-mediated leaf decomposition as well as fungal and bacterial abundances, aligned rank transformation ANOVA tests were used instead. To simplify the comparisons and statistical testing, the very low nutrient level at 0 µg fungicides/L was set as control for P-communities, while for W- and V-communities the control was set at the low nutrient level and 0 µg fungicides/L, due to measured higher nutrient background levels at the sampling sites where W- and V-communities were obtained from (seeTable S1).

For multivariate data (i.e., fungal community composition), to compare fungal communities from each exposure treatment at the species level, a presence-absence table (1/0; Table S7) was generated and non-metric multidimensional scaling plots (NMDS; Clarke, 1993) were generated using the Jaccard coefficient. The assumption of homogeneous within-group dispersion was tested using the "betadisper" function within the R-package "vegan". Subsequently, a factorial permutational multivariate analysis of variance (PERMANOVA, Anderson et al., 2005) was performed on the original data with 999 permutations to assess the individual and combined effects of the independent variables ("history", "fungicide", and "nutrient"), applying the Jaccard coefficient (Real et al., 1996) as a distance measure between groups. Statistics were conducted and figures were prepared using R version 4.2.1 (R Core Team, 2022) as well as the add-on packages "vegan" (Oksanen et al., 2009), "ggplot2" and "ggh4x" (Wickham, 2016), "tidyr" (Wickham, Vaughan, et al., 2023), "dplyr" (Wickham, François, et al., 2023), "rstatix" (Alboukadel, 2023), "visreg" (Breheny & Burchett, 2017) and "ARTool" (Kay et al., 2021). The graphical abstract and Fig. 2 and 3 were created in Biorender.com. Note that the term "significant(ly)" refers to statistical significance ($p<0.05$) throughout the study.

RESULTS & DISCUSSION

Contrary to our first hypothesis (i), increasing fungicide concentrations ($p>0.05$; Fig. 4; Table 1) did not affect microbially-mediated leaf decomposition. Instead, P- and W-communities seemed to benefit from fungicide exposure at 30 and 300 µg/L (Fig. S1), observed as non-significant 30% increases in leaf decomposition rates compared to the respective fungicide-free controls (Table S10). The effect of fungicides was not reflected in the microbial communities' relative investment in degrading recalcitrant carbon (i.e., recalcitrance

ratio; Table S4), which was not significantly affected by the factors “history” and “fungicide” (p>0.4; Table 1 & S10), opposing our second hypothesis (ii). In support of our third (iii) and partially contradicting our fourth (iv) hypotheses, increasing levels of nutrients tended to buffer for the non-significant fungicide-induced effects on leaf decomposition compared to fungicide-free treatments (Fig. S1; Table S10). Additionally, fungal community composition was significantly changed by increasing fungicide concentrations (see below). However, changes in the fungal community structure seems decoupled from its function, represented by leaf decomposition (see Feckler & Bundschuh, 2020).

Effects of fungicides on microbial communities with differing exposure histories.

In addition to the positive effects on leaf decomposition of communities from P- and W-streams, fungicides induced significant effects on the leaf-associated microbial community structure, namely on bacterial and fungal abundances (both p<0.01; Table 1), which have also been reported elsewhere (e.g., Feckler et al, 2018; Fernández et al., 2015). The bacterial and fungal abundances showed no significant changes at low to intermediate fungicide

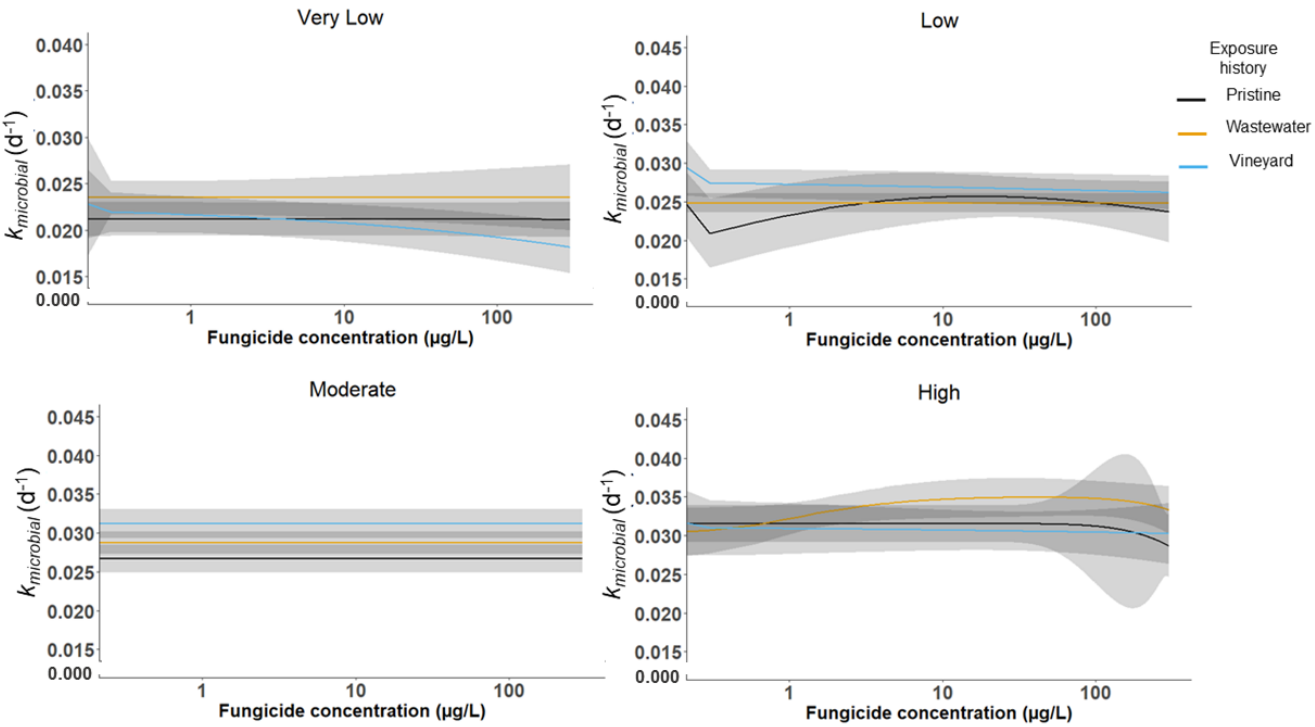

Figure 4. Dose-response models for the microbial breakdown rate ($k_{microbial}$ (d^{-1})) as a function of the total fungicide concentration (log10 scale), displayed separately for the four different nutrient levels. Shaded lines indicating corresponding 95% confidence bands (n = 5).

concentrations (3 and 30 µg/L; Fig. S5 & S6; Table 1; S1 & S12; p<0.05) compared to the respective controls. While in Fernández et al, (2015) bacterial density tended to increase in vineyard impacted sites. However, across all fungicide concentrations, the abundances were

consistently lower in the V-community compared to the equivalent treatment in the W- and P-communities (Table 1; S10; S11& S12). Moreover, the high fungicide concentration (300 µg/L) negatively affected fungal abundances, reflected in an up to 60% reduced fungal abundance independent of the history or nutrient level ($p<0.05$; Table 1; S11 & S12; Fig. S6).

Besides impacts on fungal abundance, fungal communities of the control and lower fungicide concentrations (0 and 3 µg/L) showed considerable similarity, while a substantial difference relative to the highest fungicide concentration was uncovered – a pattern observed across all nutrient levels ($p=0.001$; Fig. 5). The same pattern among fungicide concentrations was also reported in terms of fungal taxa richness (Fig. S7, S8 & S9). Moreover, fungal community composition differed among exposure histories ($p=0.001$, Table 1). Thus, these observations partially contradict the hypothesised link between the fungal community structure and their function (hypothesis iv), as we expected to see an effect on the function leaf decomposition based on the diversity and abundance changes of the fungal species within the community. Our results are pointing towards functional stability despite community shifts (reviewed in Feckler & Bundschuh, 2020). Functional stability could be achieved due to functional similarity (Eisenhauer et al., 2023) within microbial communities and an increase in the dominance of tolerant fungal species that are at the same time more efficient in leaf decomposition (Ferreira & Chauvet, 2012; Pascoal et al., 2005). This assumption is supported by the NGS data, since in most of the cases tolerant AH species of the genus *Tetracladium (T. marchalianum, T. breve, T. setigerum)* with a superior leaf decomposition efficiency (e.g., Andrade et al., 2016; Duarte et al., 2006; Zubrod et al., 2015) dominated at high fungicide exposure independent of exposure history (Table S7). Besides the increasing relevance of the genus *Tetracladium,* the species *Lemonniera terrestris, Flagellospora curvula*, and *Fusarium oxysporum* were more frequently detected with increasing fungicide concentrations. While those species are considered tolerant, knowledge on their traits is limited and partly contradicting, hampering a mechanistic interpretation (Bundschuh et al., 2011; Pascoal et al., 2005). Nonetheless, Bundschuh et al. (2011) *found F. curvula* to be most abundant under control conditions with decreasing appearance at higher fungicide concentrations. In contrast, we found this species most frequently in presence of fungicides. The opposite pattern is observed for *C. aquatica*: Pascoal et al. (2005) frequently detected this species in polluted streams of Northern Portugal, whereas we found this species more frequently in the absence of fungicides suggesting phenotypic plasticity (e.g., Quainoo et al., 2016). Notwithstanding, our findings support the principle of stable functioning being mediated by the dominance of highly efficient decomposers. These results are supported by other studies (reviewed in Feckler &

Table 1. Output for statistical analyses, namely aligned ranks transformation ANOVA for microbial leaf decomposition as well as bacterial and fungal abundance (respective post-hoc testing in Table S11), ANOVA for recalcitrance ratio, and PERMANOVA for fungal community composition. Df, degrees of freedom; Df res, residual degrees of freedom for each model; F value, ratio of variances; SE, standard error of the estimate; SS, sum of squares. p-values printed in bold indicate statistical significance.

Variable	Source of variation	Df	SS	Df res	F-value	p-value
Leaf decomposition	Fungicide	3	-	592	0.367	0.776
	Nutrient	**3**	**-**	**592**	**70.938**	**<0.001**
	History	**2**	**-**	**592**	**6.592**	**0.001**
	Fungicide x Nutrient	9	-	592	1.446	0.164
	Fungicide x History	6	-	592	1.151	0.330
	Nutrient x History	**6**	**-**	**592**	**3.100**	**0.005**
	Fungicide x Nutrient x History	18	-	592	0.268	0.999
Bacterial abundance	**Fungicide**	**3**	-	**336**	**8.204**	**<0.001**
	Nutrient	3	**-**	336	1.839	0.139
	History	**2**	**-**	**336**	**4.009**	**0.019**
	Fungicide x Nutrient	9	**-**	336	0.854	0.566
	Fungicide x History	6	**-**	336	0.202	0.975
	Nutrient x History	**6**	**-**	**336**	**3.059**	**0.006**
	Fungicide x Nutrient x History	18	**-**	336	1.186	0.269
Fungal abundance	**Fungicide**	**3**	-	**336**	**7.499**	**<0.001**
	Nutrient	3	**-**	336	1.888	0.131
	History	**2**	**-**	**336**	**3.089**	**0.046**
	Fungicide x Nutrient	9	-	336	1.013	0.428
	Fungicide x History	6	**-**	336	0.234	0.965
	Nutrient x History	**6**	**-**	**336**	**4.255**	**<0.001**
	Fungicide x Nutrient x History	18	-	336	1.318	0.173
Recalcitrance ratio	Fungicide	1	<0.001	<0.001	0.003	0.958
	Nutrient	3	<0.001	<0.001	0.483	0.697
	History	2	<0.001	<0.001	0.943	0.403
	Fungicide x History	2	<0.001	<0.001	0.560	0.579
	Nutrient x History	6	<0.001	<0.001	0.231	0.962
	Fungicide x Nutrient	3	<0.001	<0.001	0.164	0.919
	Fungicide x Nutrient x History	6	<0.001	<0.001	0.324	0.918
	Residuals	24	0.002	<0.001		
Community composition	**Fungicide**	**1**	**2.738**	**0.127**	**11.145**	**0.001**
	Nutrient	**2**	**0.711**	**0.033**	**1.447**	**0.034**
	History	**2**	**1.340**	**0.062**	**2.728**	**0.001**
	Fungicide x Nutrient	2	0.571	0.026	1.163	0.208
	Fungicide x History	**2**	**0.753**	**0.035**	**1.533**	**0.018**
	Nutrient x History	4	1.105	0.051	1.124	0.197
	Fungicide x Nutrient x History	4	0.974	0.045	0.991	0.485
	Residual	54	13.267	0.618		
	Total	71	21.462	1		

Bundschuh 2020), pointing to a maintained functional performance (i.e., leaf decomposition) when the microbial community is dominated by a few species with superior traits that compensate biodiversity loss (Dangles & Malmqvist, 2004).

Effects of nutrients on microbial communities with differing exposure histories.

Leaf decomposition significantly benefited from increasing nutrient concentrations (hypothesis iii), while the effect strength depended on the exposure history (p=0.005; Table 1). Especially at moderate and high nutrient levels, leaf decomposition increased by up to 30%, 18% and 7% for P-, W- and V-communities (Table S10), respectively, relative to the respective control scenarios (Table S10; Fig. 4). These observations may be explained by the dynamic energy budget theory (Kooijman, 2000), namely that the ease of accessing nutrients from the medium supports microbial growth and thus the functional performance as more energy is available for producing exoenzymes needed for leaf degradation (Bärlocher & Corkum, 2003). This assumption is also supported by Feckler et al. (2018), who studied equivalents to the P- and V-communities assessed here, observing higher leaf decomposition in treatments with higher nutrient availability (see also Pascoal & Cássio, 2004; Suberkropp et al., 2010). Thus, we assume that in ecosystems with higher nutrient inputs, changes in the function due to

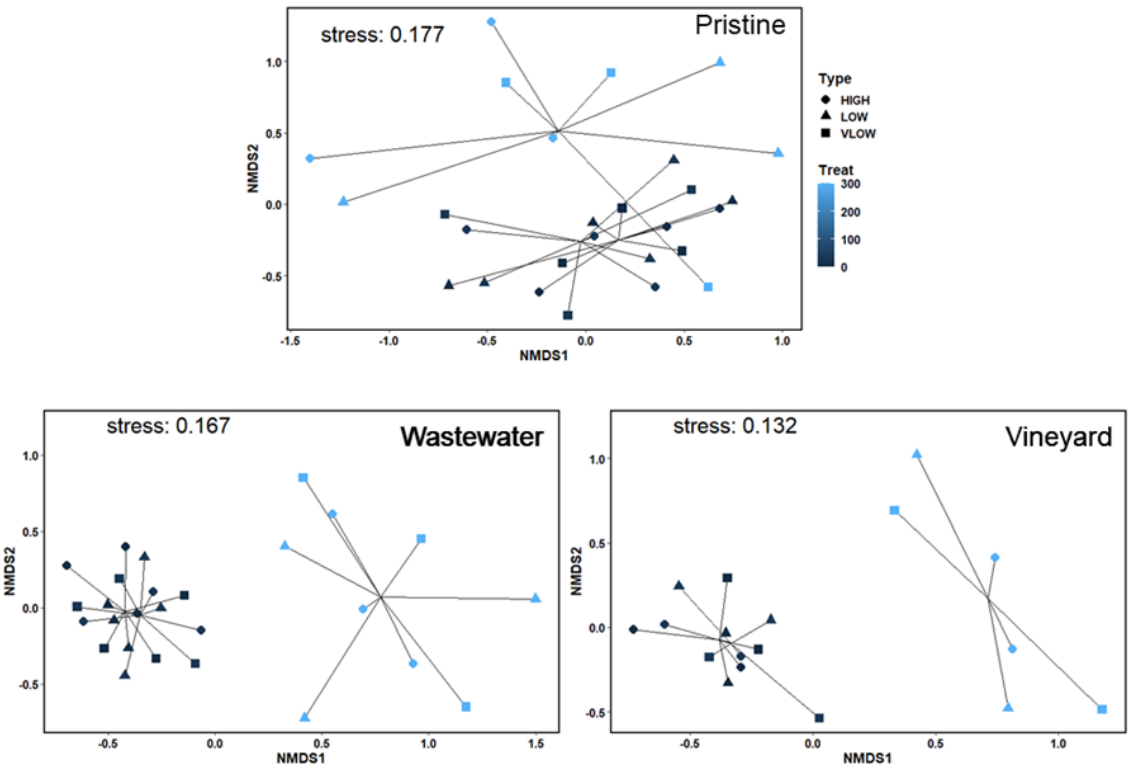

Figure 5. Non-metric multidimensional scaling (NMDS) plots for leaf-associated aquatic hyphomycete communities originating from streams with differing land-use in their catchments (Pristine, Wastewater treatment plants, Vineyard). Nutrient levels are indicated by symbols: very low= squares, low= triangles, high = circles. Colours indicate fungicide concentrations: 0 µg/L and 30 µg/L = dark blue, 300 µg/L = light blue. Spider webs connect the samples of each treatment at their respective group centroid. The stress value is provided as a measure of "goodness-of-fit" for NMDS, with a reasonable fit indicated when below 0.2 (Clarke, 1993).

chemical stress exposure being less pronounced due to “free” energy from the available nutrients (see Rossi et al., 2018 but also see Fernández et al., 2016).

Despite the positive effect of nutrients on leaf decomposition, microbial abundances were significantly affected by exposure history, with P-communities being characterised by up to 20-fold higher bacterial and fungal abundances compared to W- and V-communities within the same nutrient level (Table S10, S11 & S12; Fig. S5 & S6). Contrary to the structural parameters, the leaf decomposition performed by W-communities was slightly (up to 15%) but significantly ($p < 0.003$) higher in comparison to the P-communities, while in V-communities the function was up to 40% significantly lower than in P-communities ($p < 0.01$; Tables 1 & S10, Fig. 4). This observation may be an experimental artefact since the proxies used for microbial abundances (bacterial and fungal) do not account for changes in the fungal community composition and consequently its composition in terms of functional traits (Englert et al., 2015; Rossi et al., 2018). It may be that microbes characterised by a high leaf decomposition efficiency dominate over those with a lower efficiency capable of maintaining the function (e.g., Reiss et al., 2010).

Combining chemical stressors and exposure history.

Our study found changes in community structures at high fungicide exposure across all exposure histories. We expected more pronounced effects of fungicides on P-communities compared to communities with exposure history (W- and V-communities). This expectation was not met, potentially due to the presence of some tolerant species, such as *T. marchalianum*, also in P-communities. The latter could also have happened due to the relatively low fungicide concentrations used here compared to other studies. Although sum fungicide concentrations of 300 µg/L are above the high end of environmentally relevant concentration ranges (Landesamt für Umwelt, 2016; e.g. sum pesticide concentrations measured during rainfall events went up to 83.4 µg/L in Bereswill et al., 2022), these levels have been too low to obtain more pronounced responses in leaf decomposition and community structure during laboratory studies (see Feckler et al., 2017; Gonçalves et al., 2023; Zubrod, et al., 2015). Under field conditions, however, lower concentrations of fungicides contributed to changes on the fungal community structure (e.g., Fernández et al., 2016). Moreover, the high variability and non-consistent patterns found among our three bioassays could be explained by the different sampling season and the respective naturally differing enzyme activities (Bastias et al., 2022). The latter suggests that the local community and potentially the colonisation dynamics play a significant role, which should be further and individually studied (Mora-Gómez et al., 2016).

CONCLUSION

Overall, the present study shows that leaf decomposition was not affected by increasing fungicide concentrations and “fungicides” or “history” did not affect that degradation of recalcitrant carbon by microbial communities. While increasing levels of nutrients tended to buffer the non-significant fungicide-induced effects on leaf decomposition. The presence of higher nutrient levels eased the access to nutrients supporting microbial growth and functional performance, as more energy is available for producing exoenzymes needed for leaf degradation. Moreover, increasing fungicide concentrations changed significantly the fungal community composition across all the exposure histories. The changes found on their structure seemed decoupled from its function, represented by leaf decomposition, which points towards functional stability despite community shifts. The changes in fungal species composition in this and previous studies, suggest phenotypic plasticity and supporting the principle of stable functioning being mediated by the dominance of highly efficient decomposers. This fewer species with superior traits maintain functional performance while compensating biodiversity loss. Additionally, future studies should further assess local communities and potentially the colonisation dynamics role in response to nutrient and fungicide stressors.

In conclusion, our study points to the benefits of a combined assessment of ecosystem structure and function, which not only supports the interpretation of the data but also fuels the research field related to the link between biodiversity and ecosystem function – particularly in the context of chemical stressors. The changes in the fungal community composition under fungicide exposure despite functional stability raises potential concerns, as in case only functional measures are used to assess environmental impacts, structural changes remain unnoticed. This concern is informed by the key role of aquatic fungi in ecosystems, which is regulating aquatic food webs in a bottom-up direction (Arsuffi & Suberkropp, 1989; Gonçalves et al., 2014). Fungal species considered tolerant are often not only rejected by but also not as nutritional for shedders, which can potentially influence their fitness and development (e.g., Gonçalves et al., 2023b).As our mechanistic understanding of this bottom-up regulation is limited, future research is needed, including the consideration of fungal traits under multiple stress scenarios (Loreau et al., 2001)

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Supplementary information:

Elevated Fungicide and Nutrient Concentrations Change Structure but not Function of Aquatic Microbial Communities.

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A1- Tables and figures

Table S1 - Information on sampling sites, location, date of sampling and water parameters: pH, Temperature, Conductivity, Oxygen, NO_3, PO_4.

Site	land-use category	Parameters	Colonization start date	Technical check middle colonization	End Colonization date
			11.04.19	18.04.19	25.04.19
P1	P	pH	7.64	7.37	7.89
Hainbach		Temperature (ºC)	8.9	8.2	11.7
49.240786, 8.046816		conductivity (µS/cm)	123	119	123
		O_2 (%)	96.47	111	128
		O_2 (mg/L)	10.79		11.87
		NO_3 (mg/L)	0.2-0-7	0.2-0-7	0-0.2
		PO_4 (mg/L)	<0.15	<0.15	0.46
		P_2O_5 (mg/L)	<0.11	<0.11	0.34
W1	W	pH	7.34	7.192	7.28
Queich		Temperature (ºC)	12.5	12.5	14.6
49.204169, 8.190974		Conductivity (µS/cm)	604	483	456
		O_2 (%)	102.4	138.3	135
		O_2 (mg/L)	10.7		13.31
		NO_3 (mg/L)	1.1 - 2.3	1.1	1.1
		PO_4 (mg/L)	0.15	0.15	0.15 - 0.31
		P_2O_5 (mg/L)	0.11	<0.11	0.11 - 0.23
V1	V	pH	7.75	7.68	7.68
Hainbach		Temperature (ºC)	12.9	10.3	14.6
49.236277, 8.075976		Conductivity (µS/cm)	196	185.1	186
		O_2 (%)	139	117.9	125
		O_2 (mg/L)	14.65		12.34
		NO_3 (mg/L)	0.7-1.1	0.7-1.1	0.7
		PO_4 (mg/L)	0.15	<0.15	0.15 - 0.31
		P_2O_5 (mg/L)	0.11	<0.11	0.11 - 0.23
		Date	30.05.19	06.06.19	13.06.19
P2	P	pH	7.52	7.57	7.55
Eußerbach		Temperature (ºC)	10.1	11	12.7
49.257339, 7.960379		Conductivity (µS/cm)	83	84	84
		O_2 (%)	150	208	151.4
		O_2 (mg/L)		19.75	19.29
		NO_3 (mg/L)	10	04-10	10
		PO_4 (mg/L)	0.0-0.05	0	0
W2	W	pH	7.55	7.34	7.33
Triefenbach :		Temperature (ºC)	15.1	18	19.2
49.282329, 8.164092		Conductivity (µS/cm)	496	633	442

		O_2 (%)	90	102	92
		O_2 (mg/L)		12.24	8.3
		NO_3 (mg/L)	10	10-20	20-30
		PO_4 (mg/L)	0.1-0.15	0.1	0.05
V2	V	pH	8.06	8.03	8.28
Modenbach		Temperature (ºC)	12.6	15.2	15.5
49.258726, 8.118499		Conductivity (µS/cm)	400	363	397
		O_2 (%)	134	109.3	129.1
		O_2 (mg/L)		10.8	11.88
		NO_3 (mg/L)	20	10-20	20
		PO_4 (mg/L)	0.05	0.05	0.05
		Date	27.08	3.09	10.09
P3	P	pH	7.78	7.89	7.97
Heiderbrunnertalbach		Temperature (ºC)	18.1	12.1	11.6
49.355616,8.095295		Conductivity (µS/cm)	176	181	184
		O_2 (%)	87	94.5	100.1
		O_2 (mg/L)	8.1	10.1	10.69
		NO_3 (mg/L)	0.2-0-7	0-0.2	0.2-0-7
		PO_4 (mg/L)	0.15	<0.15	<0.15
		P_2O_5 (mg/L)	0.11	<0.11	<0.11
W3	W	pH	7.43	7.57	7.61
Speyerbach		Temperature (ºC)	21.9	14	13.1
49.325734,8.245539		Conductivity (µS/cm)	196	184	193
		O_2 (%)	84	83.7	83.9
		O_2 (mg/L)	7.2	8.6	8.77
		NO_3 (mg/L)	1.1-2.3	0.7-1.1	1.1
		PO_4 (mg/L)	0.15 - 0.31	0.15	0.15 - 0.31
		P_2O_5 (mg/L)	0.11 - 0.23	0.11	<0.11
V3	V	pH	7.63	DRY	7.55
Schlittgraben		Temperature (ºC)	20.1		13.3
49.32044255691864, 8.160387527092078		Conductivity (µS/cm)	453		431
		O_2 (%)	39.5		37.4
		O_2 (mg/L)	3		3.91
		NO_3 (mg/L)	2.3-4.0		2.3-4
		PO_4 (mg/L)	0.38		0.15 - 0.31
		P_2O_5 (mg/L)	0.23		0.11 - 0.23

Table S1 continuation

Table S2 – Information on the fungicide mixture, their product names, manufacturers, active ingredient concentrations, nominal test concentrations (used in this study as a mixture), and mode of action.

Substance	Product name	Manufacturer	Concentration active ingredients in formulation /product	Nominal test concentrations (µg/L)	Mode of action - Fungicide Resistance Action Committee (2017)
Azoxystrobin	Ortiva	Syngenta Agro	250 g/L	0; 0,5; 5; 50	Inhibition of mitochondrial respiration
Carbendazim	Derosol	Bayer Crop Science	600 g/kg	0; 0,5; 5; 50	Inhibition of mitosis and cell division
Cyprodinil	Chorus	Syngenta Agro	500 g/kg	0; 0,5; 5; 50	Inhibition of amino acid and protein synthesis
Quinoxyfen	Fortess 250	Dow Agro Science	250 g/L	0; 1; 10; 100	Perturbation of signal transduction
Tebuconazole	Folicur	Bayer Crop Science	250 g/L	0; 0,5; 5; 50	Inhibition of sterol biosynthesis
Mixture of all above				0; 3; 30; 300	

Table S3 – Measured and nominal fungicide concentrations along the assays, excluding Quinoxyfen, which was not measured due to high residuals. (LOQ – limit of quantification; Initial- initial fungicide spike sampling; initial + 2h- sampling after 2h of spiking; 7d- sampling after 7days).

Time	Tebuconazole [µg/L]	Azoxystrobin [µg/L]	Carbendazim [µg/L]	Cyprodinil [µg/L]	Sum measured	Nominal sum concentration	Variation %
Initial	< LOQ	< LOQ	< LOQ	< LOQ	0	0	0
Initial + 2h	< LOQ	< LOQ	< LOQ	< LOQ	0	0	0
7d	< LOQ	< LOQ	< LOQ	< LOQ	0	0	0
Initial	< LOQ	< LOQ	0.549	0.446	0.988	2	0.505
Initial + 2h	< LOQ	< LOQ	0.412	0.346	0.758	2	0.620
7d	< LOQ	< LOQ	0.344	< LOQ	0.344	2	0.828
Initial	6.323	6.793	6.802	2.981	22.900	20	-0.145
Initial + 2h	4.104	4.296	4.751	1.992	15.145	20	0.242
7d	5.180	4.683	4.996	0.876	15.736	20	0.213
Initial	59.623	52.541	48.480	26.725	187.370	200	0.063
Initial + 2h	44.007	38.402	41.717	16.550	140.677	200	0.296
7d	52.084	40.260	45.367	15.422	153.134	200	0.234

Table S4 – Investment in recalcitrant carbon degradation calculated as the ratio of oxidases divided by total hydrolases using square-root transformed data. The lower the ratio the higher the relative investment in recalcitrant carbon degradation.

Community history		**Pristine**	**Wastewater**	**Vineyard**
Sum fungicide concentration µg/L	**Nutrient levels**	**Recalcitrance ratio**		
0	Very Low	0.060	0.049	0.056
	Low	0.046	0.049	0.059
	Mod	0.058	0.061	0.070
	High	0.057	0.059	0.059
3	Very Low	0.062	0.066	0.065
	Low	0.053	0.054	0.053
	Mod	0.060	0.045	0.062
	High	0.063	0.044	0.049
30	Very Low	0.062	0.062	0.073
	Low	0.088	0.077	0.061
	Mod	0.058	0.074	0.065
	High	0.064	0.062	0.054
300	Very Low	0.071	0.050	0.068
	Low	0.058	0.054	0.061
	Mod	0.061	0.049	0.061
	High	0.054	0.056	0.065

Table S5 - Information on qPCR assay developed by Manerkar et al. (2008): Targeted group, primers (Baker & Cowan, 2003; White et al., 1990) used including the template sequences as well as technical properties including melting temperature, amplified region and length (bp).

Target	Primer	Sequence	Melting temperature (ºC)	Amplified region	Amplicon length (bp)
Fungi	ITS3F	GCATCGATGAAGAACGCAGC	55.3	5.8S and ITS2	400
	ITS4R	TCCTCCGCTTATTGATATGC			
Bacteria	E8F	AGAGTTTGATCCTGGCTCAG	55	16S	525
	E533R	TIACCGIIICTICTGGCAC			

Manerkar, M. A., Seena, S., & Bärlocher, F. (2008). Q-RT-PCR for assessing archaea, bacteria, and fungi during leaf decomposition in a stream. Microbial Ecology, 56(3), 467–473. https://doi.org/10.1007/s00248-008-9365-z~

Baker GC, Smith JJ, Cowan DA. Review and re-analysis of domain-specific 16S primers. J Microbiol Methods. 2003 Dec;55(3):541-55. doi: 10.1016/j.mimet.2003.08.009. PMID: 14607398

White, T.J., Bruns, T.D., Lee, S.B. and Taylor, J.W. (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., Eds., PCR Protocols: A Guide to Methods and Applications, Academic Press, New York, 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Table S6 – Species name curation from blast (Genbank), including genbank accession number.

	Site	G	A	G	A	G	A	D	D	D	G	D	D	S
Species	**GenBank**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**
Clavariopsis aquatica	KJ170982	S1												
	LC472491	S1						S7	S8	S9		S11	S12	
	MK353102	S1					S6							S13
	GQ411316													
	MK353101	S1	S2			S5			S8	S9	s10	S11		S13
	MH047194			S3	S4	S5					S10		S12	S13
	GQ411318	S1												
Clavatospora longibrachiata	MK353105	S1				S5			S8			S11		
	MK353104										S10			
	KF730808			S3					S8	S9			S12	S13
Aquanectria penicillioides	KM231743	S1												
Stenocladiella neglecta	KX858624	S1	S2	S3	S4	S5	S6		S8	S9	S10	S11		S13
Cylindrocladiella parva	MF440366	S1		S3										
Triscelophorus cf. acuminatus	KF730835	S1												
Sydowia polyspora	LR875280	S1												
Amniculicola guttulata	MT627726	S1	S2	S3				S7	S8	S9	S10	S11	S12	S13
Lunulospora curvula	OK605579	S1		S3		S5					S10			
	OK605578													
Colispora cavincola	MH862544	S1				S5								S13
Lemonniera cornuta	OM907741	S1						S7						
	KU519115													
	KX858620													
Alatospora acuminata	AY204590	S1		S3	S4	S5	S6		S8		S10	S11		S13
	MK353091		S2		S4	S5					S10		S12	S13
	MH930815		S2		S4								S12	
	MK353089		S2		S4	S5	S6							S13
	AY204587				S4									
	KX858600				S4		S6							S13
	MK353087				S4		S6							S13
	MK353088										S10			
	AY204589											S11		
	MK353090						S6							
	MK353092	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Tetrachaetum elegans	KF952682	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
	KX858625							S7						
Tricladium chaetocladium	KC834067	S1		S3				S7	S8	S9		S11		
	MZ773531													
Tetracladium marchalianum	MH930823	S1									S10			
	KF952709													
	AY204624													
	KX858642													
	MN459681										S10			
	LR875991					S5								
	LR875992													
	MK353124													
	MK353125		S2										S12	
	MK353126		S2	S3				S7	S8					
	MK353127		S2									S11		

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	G	A	A	A	G	A	D	D	A	G
Species	**GenBank**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	**S23**	**S24**	**S25**
Clavariopsis aquatica	KJ170982												
	LC472491	S14	S15							S22			
	MK353102												
	GQ411316	S14											
	MK353101		S15	S16	S17	S18							
	MH047194												
	GQ411318	S14											
Clavatospora longibrachiata	MK353105	S14	S15										
	MK353104												
	KF730808		S15	S16									
Aquanectria penicillioides	KM231743												S25
Stenocladiella neglecta	KX858624	S14	S15	S16									
Cylindrocladiella parva	MF440366					S18		S20	S21			S24	
Triscelophorus cf. acuminatus	KF730835	S14											
Sydowia polyspora	LR875280												
Amniculicola guttulata	MT627726	S14	S15	S16									
Lunulospora curvula	OK605579	S14											
	OK605578												
Colispora cavincola	MH862544	S14											
Lemonniera cornuta	OM907741												
	KU519115												
	KX858620												
Alatospora acuminata	AY204590			S16	S17	S18							
	MK353091				S17		S19						
	MH930815				S17								
	MK353089		S15										
	AY204587				S17								
	KX858600					S18							
	MK353087												
	MK353088												
	AY204589												
	MK353090												
	MK353092	S14	S15	S16	S17	S18		S20		S22	S23		
Tetrachaetum elegans	KF952682	S14	S15	S16	S17	S18		S20	S21	S22	S23	S24	S25
	KX858625						S19						
Tricladium chaetocladium	KC834067	S14	S15	S16									
	MZ773531												
Tetracladium marchalianum	MH930823		S15	S16							S23		
	KF952709												
	AY204624												
	KX858642												
	MN459681			S16									S25
	LR875991												
	LR875992								S21				S25
	MK353124												
	MK353125												
	MK353126	S14						S20	S21	S22			S25
	MK353127												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	C	F	C	C	F	F	C	F	B	H	H	B
Species	**GenBank**	**S38**	**S39**	**S40**	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S47**	**s48**	**S49**
Clavariopsis aquatica	KJ170982												
	LC472491		S39							S46		s48	
	MK353102												
	GQ411316												
	MK353101	S38	S39							S46	S47		S49
	MH047194		S39								S47		
	GQ411318								S45	S46			
Clavatospora longibrachiata	MK353105										S47		S49
	MK353104												
	KF730808		S39							S46	S47	S48	
Aquanectria penicillioides	KM231743											S48	
Stenocladiella neglecta	KX858624	S38	S39							S46	S47	S48	S49
Cylindrocladiella parva	MF440366			S40					S45		S47	S48	S49
Triscelophorus cf. acuminatus	KF730835											S48	
Sydowia polyspora	LR875280						S43		S45				
Amniculicola guttulata	MT627726	S38	S39		S41					S46	S47	S48	S49
Lunulospora curvula	OK605579									S46	S47	S48	
	OK605578												
Colispora cavincola	MH862544	S38								S46		S48	S49
Lemonniera cornuta	OM907741			S40									
	KU519115												
	KX858620												
Alatospora acuminata	AY204590	S38									S47		
	MK353091	S38								S46	S47		S49
	MH930815												S49
	MK353089	S38											
	AY204587	S38											
	KX858600												
	MK353087	S38								S46			
	MK353088												
	AY204589												
	MK353090												
	MK353092	S38	S39			S42			S45	S46	S47		S49
Tetrachaetum elegans	KF952682	S38	S39	S40	S41	S42	S43		S45		S47	S48	S49
	KX858625												
Tricladium chaetocladium	KC834067		S39									S48	S49
	MZ773531												
Tetracladium marchalianum	MH930823	S38	S39	S40	S41	S42	S43	S44	S45	S46			S49
	KF952709												
	AY204624		S39										
	KX858642			S40									
	MN459681	S38							S45				
	LR875991												
	LR875992												
	MK353124		S39	S40		S42	S43		S45				S49
	MK353125	S38		S40		S42	S43	S44	S45	S46			S49
	MK353126	S38									S47		
	MK353127												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	C	C	F	F	C	F	F	C	F	C
Species	**GenBank**	**S26**	**S27**	**S28**	**S29**	**S30**	**S31**	**S32**	**S33**	**S34**	**S35**	**S36**	**S37**
Clavariopsis aquatica	KJ170982												
	LC472491		S27			S30	S31		S33				
	MK353102												
	GQ411316												
	MK353101	S26		S28	S29	S30	S31	S32	S33	S34		S36	S37
	MH047194							S32					
	GQ411318					S30							
Clavatospora longibrachiata	MK353105							S32	S33				
	MK353104												
	KF730808			S28		S30						S36	
Aquanectria penicillioides	KM231743	S26											
Stenocladiella neglecta	KX858624			S28	S29		S31		S33	S34			
Cylindrocladiella parva	MF440366								S33				
Triscelophorus cf. acuminatus	KF730835												
Sydowia polyspora	LR875280												
Amniculicola guttulata	MT627726		S27	S28	S29	S30	S31	S32	S33	S34		S36	S37
Lunulospora curvula	OK605579					S30	S31					S36	
	OK605578					S30			S33				
Colispora cavincola	MH862544												
Lemonniera cornuta	OM907741		S27			S30			S33			S36	
	KU519115												
	KX858620												
Alatospora acuminata	AY204590			S28				S32					S37
	MK353091			S28	S29	S30	S31	S32		S34	S35	S36	S37
	MH930815							S32				S36	S37
	MK353089			S28				S32					S37
	AY204587			S28							S35		
	KX858600												
	MK353087			S28		S30		S32					S37
	MK353088												
	AY204589												
	MK353090												
	MK353092		S27	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37
Tetrachaetum elegans	KF952682	S26	S27	S28	S29	S30	S31	S32	S33		S35	S36	S37
	KX858625												
Tricladium chaetocladium	KC834067		S27										
	MZ773531												
Tetracladium marchalianum	MH930823			S28	S29	S30	S31		S33		S35	S36	S37
	KF952709												
	AY204624						S31		S33				
	KX858642												
	MN459681	S26			S29								
	LR875991												
	LR875992	S26											
	MK353124						S31					S36	
	MK353125				S29	S30					S35	S36	
	MK353126	S26	S27	S28		S30	S31	S32	S33	S34		S36	
	MK353127				S29							S36	

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	E	B	E	E	H	E	B	B	H	B	E	E
Species	**GenBank**	**S50**	**S51**	**S52**	**S53**	**S54**	**S55**	**S56**	**S57**	**S58**	**S59**	**S60**	**S61**
Clavariopsis aquatica	KJ170982		S51										
	LC472491		S51			S54	S55				S59		
	MK353102												
	GQ411316												
	MK353101	S50	S51	S52	S53	S54	S55	S56	S57		S59	S60	S61
	MH047194		S51										
	GQ411318	S50			S53	S54		S56		S58	S59		
Clavatospora longibrachiata	MK353105	S50	S51			S54	S55	S56		S58		S60	S61
	MK353104												
	KF730808			S52	S53	S54	S55		S57		S59		
Aquanectria penicillioides	KM231743					S54				S58			
Stenocladiella neglecta	KX858624		S51		S53	S54	S55	S56	S57	S58			S61
Cylindrocladiella parva	MF440366	S50			S53					S58			
Triscelophorus cf. acuminatus	KF730835												
Sydowia polyspora	LR875280												S61
Amniculicola guttulata	MT627726	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60	S61
Lunulospora curvula	OK605579		S51		S53	S54				S58			
	OK605578												
Colispora cavincola	MH862544	S50	S51			S54		S56		S58	S59		S61
Lemonniera cornuta	OM907741						S55						
	KU519115		S51										
	KX858620												
Alatospora acuminata	AY204590		S51						S57		S59		
	MK353091		S51		S53			S56	S57		S59		S61
	MH930815												
	MK353089												
	AY204587												
	KX858600												
	MK353087												
	MK353088		S51										
	AY204589												
	MK353090												
	MK353092	S50	S51		S53	S54	S55	S56	S57	S58	S59	S60	S61
Tetrachaetum elegans	KF952682	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60	S61
	KX858625												
Tricladium chaetocladium	KC834067		S51								S59		
	MZ773531												
Tetracladium marchalianum	MH930823		S51		S53		S55	S56	S57	S58	S59	S60	S61
	KF952709								S57				
	AY204624												
	KX858642												
	MN459681								S57				
	LR875991						S55						
	LR875992												
	MK353124								S57				
	MK353125	S50		S52	S53	S54	S55		S57		S59		
	MK353126												
	MK353127												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	H	H	E	B	B	B	E	H	H	E	H
Species	**GenBank**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Clavariopsis aquatica	KJ170982											
	LC472491		S63									
	MK353102											
	GQ411316											
	MK353101									S70		
	MH047194	S62										
	GQ411318		S63									S72
Clavatospora longibrachiata	MK353105											
	MK353104											
	KF730808											
Aquanectria penicillioides	KM231743								S69			S72
Stenocladiella neglecta	KX858624	S62	S63									
Cylindrocladiella parva	MF440366	S62	S63		S65			S68		S70		
Triscelophorus cf. acuminatus	KF730835		S63									
Sydowia polyspora	LR875280											
Amniculicola guttulata	MT627726	S62	S63									S72
Lunulospora curvula	OK605579	S62	S63									
	OK605578											
Colispora cavincola	MH862544		S63									
Lemonniera cornuta	OM907741											
	KU519115											
	KX858620											
Alatospora acuminata	AY204590	S62										
	MK353091	S62										
	MH930815											
	MK353089											
	AY204587											
	KX858600											
	MK353087	S62										
	MK353088	S62										
	AY204589											
	MK353090											
	MK353092	S62	S63									
Tetrachaetum elegans	KF952682	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71	S72
	KX858625											
Tricladium chaetocladium	KC834067		S63									
	MZ773531				S65							
Tetracladium marchalianum	MH930823	S62		S64	S65	S66	S67	S68	S39	S70	S71	S72
	KF952709											
	AY204624					S66						
	KX858642											
	MN459681											
	LR875991											
	LR875992											
	MK353124						S67					
	MK353125						S67	S68				
	MK353126			S64		S66						
	MK353127											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	A	G	A	G	A	D	D	D	G	D	D	S
Species	**GenBank**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**
Margaritispora aquatica	MK353138	S1	S2						S8		S10			
	MK353139													
Vishniacozyma heimaeyensis	KX096666	S1												
	MK782337													
Dactylella microaquatica	MH857842		S2											
Pseudopithomyces palmicola	MT557510		S2											
	MT557249													
	MT557289				S4									
	MT557503		S2		S4	S5		S7		S9		S11		S13
Pseudopithomyces chartarum	MT420634		S2							S9		S11	S12	
	KX664331											S11		
	MT635315		S2				S6			S9				
	MH860227				S4									
	MT420626		S2							S9		S11	S12	
Amniculicola longissima	MK353143		S2	S3		S5					S10		S12	
	MN660520													
	KJ171067										S10			
	AY204595													
	MK371721		S2	S3		S5					S10			
Juxtiphoma eupyrena	MN823566		S2											
Fusarium sporotrichioides	MT635298		S2						S8					
Flagellospora curvula	MK353112		S2	S3		S5	S6	S7	S8	S9		S11		S13
	KC834050													
Articulospora tetracladia	MK353100		S2	S3	S4	S5	S6	S7	S8			S11		S13
	MK353096													
	EU998924													
	MK353099													S13
	MK353098		S2						S8					
	KU892281		S2									S11		S13
	EU998928		S2		S4			S7						S13
	KP234384		S2		S4			S7						
	EU998921		S2											
	LC131004													
	EU998915				S4									
	EU998920				S4	S5						S11		S13
	EU998927				S4									
	KP234366				S4									
	KP234369													
	JF895437													
	EU998929				S4									
	KP234371		S2		S4				S8	S9		S11		S13
Gyoerffyella entomobryoides	MH858280		S2		S4			S7						S13
	NR_145302											S11		
Lemonniera terrestris	MH930821		S2		S4		S6	S7	S8					S13
	MK353114							S7	S8	S9		S11		
Filosporella annelidica	MK353108		S2		S4									S13
	MT185424													
Lemonniera pseudofloscula	OM907742		S2											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	G	A	A	A	G	A	D	D	A	G
Species	**GenBank**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	**S23**	**S24**	**S25**
Margaritispora aquatica	MK353138	S14	S15		S17		S19	S20	S21	S22			S25
	MK353139												
Vishniacozyma heimaeyensis	KX096666												
	MK782337												
Dactylella microaquatica	MH857842												
Pseudopithomyces palmicola	MT557510												
	MT557249				S17								
	MT557289												
	MT557503		S15		S17	S18			S21	S22		S24	
Pseudopithomyces chartarum	MT420634		S15										
	KX664331		S15										
	MT635315												
	MH860227											S24	
	MT420626				S17	S18			S21		S23	S24	
Amniculicola longissima	MK353143				S17	S18							S25
	MN660520												
	KJ171067												
	AY204595											S24	
	MK371721			S16									
Juxtiphoma eupyrena	MN823566												
Fusarium sporotrichioides	MT635298												
Flagellospora curvula	MK353112		S15	S16		S18		S20		S22			
	KC834050												
Articulospora tetracladia	MK353100		S15	S16	S17	S18						S24	
	MK353096												
	EU998924				S17								
	MK353099												
	MK353098				S17	S18							
	KU892281					S18							
	EU998928				S17	S18							
	KP234384				S17	S18							
	EU998921												
	LC131004					S18							
	EU998915												
	EU998920				S17	S18							
	EU998927												
	KP234366												
	KP234369			S16									
	JF895437												
	EU998929												
	KP234371		S15	S16	S17	S18							
Gyoerffyella entomobryoides	MH858280												
	NR_145302					S18							
Lemonniera terrestris	MH930821			S16	S17	S18						S24	
	MK353114				S17						S23		
Filosporella annelidica	MK353108				S17	S18							
	MT185424												
Lemonniera pseudofloscula	OM907742												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	C	C	F	F	C	F	F	C	F	C
Species	**GenBank**	**S26**	**S27**	**S28**	**S29**	**S30**	**S31**	**S32**	**S33**	**S34**	**S35**	**S36**	**S37**
Margaritispora aquatica	MK353138	S26		S28	S29		S31	S32	S33	S34		S36	S37
	MK353139							S32					
Vishniacozyma heimaeyensis	KX096666							S32					
	MK782337												
Dactylella microaquatica	MH857842			S28	S29	S30	S31		S33	S34		S36	S37
Pseudopithomyces palmicola	MT557510												
	MT557249												
	MT557289												
	MT557503		S27	S28	S29		S31	S32	S33		S35		S37
Pseudopithomyces chartarum	MT420634			S28									
	KX664331						S31						
	MT635315												
	MH860227												
	MT420626			S28	S29	S30		S32			S35	S36	S37
Amniculicola longissima	MK353143	S26			S29	S30	S31	S32	S33	S34	S35	S36	S37
	MN660520								S33				
	KJ171067												
	AY204595					S30			S33			S36	
	MK371721								S33			S36	
Juxtiphoma eupyrena	MN823566												
Fusarium sporotrichioides	MT635298												
Flagellospora curvula	MK353112		S27	S28			S31	S32	S33		S35		
	KC834050						S31						
Articulospora tetracladia	MK353100		S27		S29			S32	S33				S37
	MK353096												
	EU998924												
	MK353099												
	MK353098												
	KU892281												
	EU998928		S27					S32					S37
	KP234384		S27										
	EU998921												
	LC131004												
	EU998915												
	EU998920												
	EU998927												
	KP234366												
	KP234369												
	JF895437												
	EU998929												
	KP234371												S37
Gyoerffyella entomobryoides	MH858280		S27										
	NR_145302		S27										
Lemonniera terrestris	MH930821		S27	S28	S29			S32			S35	S36	S37
	MK353114		S27					S32			S35	S36	
Filosporella annelidica	MK353108			S28	S29			S32					S37
	MT185424												
Lemonniera pseudofloscula	OM907742				S29								

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	C	F	C	C	F	F	C	F	B	H	H	B
Species	**GenBank**	**S38**	**S39**	**S40**	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S47**	**s48**	**S49**
Margaritispora aquatica	MK353138	S38		S40	S41	S42	S43		S45	S46	S47	S48	
	MK353139												
Vishniacozyma heimaeyensis	KX096666											S48	
	MK782337												
Dactylella microaquatica	MH857842	S38								S46			
Pseudopithomyces palmicola	MT557510												
	MT557249												
	MT557289												
	MT557503	S38		S40	S41		S43	S44		S46			
Pseudopithomyces chartarum	MT420634												
	KX664331		S39										
	MT635315												
	MH860227												
	MT420626	S38	S39	S40						S46	S47	S48	S49
Amniculicola longissima	MK353143		S39	S40						S46	S47	S48	S49
	MN660520												
	KJ171067												
	AY204595		S39								S47		
	MK371721		S39								S47		
Juxtiphoma eupyrena	MN823566												
Fusarium sporotrichioides	MT635298												
Flagellospora curvula	MK353112	S38	S39			S42			S45	S46	S47	S48	S49
	KC834050												
Articulospora tetracladia	MK353100	S38	S39							S46	S47		S49
	MK353096										S47		
	EU998924												
	MK353099												
	MK353098												
	KU892281										S47		
	EU998928	S38									S47		
	KP234384												
	EU998921												
	LC131004												
	EU998915												
	EU998920												
	EU998927												
	KP234366												
	KP234369												
	JF895437												
	EU998929												
	KP234371												
Gyoerffyella entomobryoides	MH858280												
	NR_145302												
Lemonniera terrestris	MH930821	S38			S41				S45		S47		S49
	MK353114	S38	S39								S47		
Filosporella annelidica	MK353108												
	MT185424												
Lemonniera pseudofloscula	OM907742										S47		

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	E	B	E	E	H	E	B	B	H	B	E	E
Species	**GenBank**	**S50**	**S51**	**S52**	**S53**	**S54**	**S55**	**S56**	**S57**	**S58**	**S59**	**S60**	**S61**
Margaritispora aquatica	MK353138	S50	S51	S52			S55		S57	S58			S61
	MK353139												
Vishniacozyma heimaeyensis	KX096666					S54							
	MK782337												
Dactylella microaquatica	MH857842		S51					S56	S57				
Pseudopithomyces palmicola	MT557510												
	MT557249												
	MT557289												
	MT557503	S50	S51		S53			S56	S57		S59		S61
Pseudopithomyces chartarum	MT420634												
	KX664331							S56					
	MT635315				S53								
	MH860227											S60	
	MT420626		S51	S52	S53			S56	S57		S59	S60	S61
Amniculicola longissima	MK353143	S50	S51	S52	S53	S54	S55	S56	S57		S59		S61
	MN660520												
	KJ171067												
	AY204595							S56					
	MK371721				S53		S55	S56			S59		S61
Juxtiphoma eupyrena	MN823566												
Fusarium sporotrichioides	MT635298												
Flagellospora curvula	MK353112		S51				S55	S56	S57		S59	S60	S61
	KC834050												
Articulospora tetracladia	MK353100							S56					
	MK353096												
	EU998924												
	MK353099												
	MK353098												
	KU892281												
	EU998928												
	KP234384												
	EU998921												
	LC131004												
	EU998915												
	EU998920												
	EU998927												
	KP234366												
	KP234369												
	JF895437												
	EU998929												
	KP234371							S56					
Gyoerffyella entomobryoides	MH858280												
	NR_145302												
Lemonniera terrestris	MH930821		S51	S52	S53		S55						
	MK353114						S55						
Filosporella annelidica	MK353108												
	MT185424										S59		
Lemonniera pseudofloscula	OM907742												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	H	H	E	B	B	B	E	H	H	E	H
Species	**GenBank**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Margaritispora aquatica	MK353138	S62	S63	S64			S67	S68	S39		S71	S72
	MK353139											
Vishniacozyma heimaeyensis	KX096666		S63									
	MK782337											
Dactylella microaquatica	MH857842		S63									
Pseudopithomyces palmicola	MT557510											
	MT557249											
	MT557289											
	MT557503	S62		S64								
Pseudopithomyces chartarum	MT420634											
	KX664331											
	MT635315											
	MH860227											
	MT420626	S62		S64								
Amniculicola longissima	MK353143						S67					
	MN660520											
	KJ171067											
	AY204595											
	MK371721											
Juxtiphoma eupyrena	MN823566											
Fusarium sporotrichioides	MT635298											
Flagellospora curvula	MK353112	S62	S63	S64			S67			S70	S71	
	KC834050											
Articulospora tetracladia	MK353100	S62										
	MK353096											
	EU998924											
	MK353099											
	MK353098											
	KU892281											
	EU998928											
	KP234384											
	EU998921											
	LC131004											
	EU998915											
	EU998920											
	EU998927											
	KP234366											
	KP234369											
	JF895437											
	EU998929											
	KP234371											
Gyoerffyella entomobryoides	MH858280											
	NR_145302											
Lemonniera terrestris	MH930821	S62								S70		
	MK353114	S62										
Filosporella annelidica	MK353108	S62										
	MT185424											
Lemonniera pseudofloscula	OM907742											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	A	G	A	G	A	D	D	D	G	D	D	S
Species	**GenBank**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**
Tricladium angulatum	MH930824			S3		S5					S10		S12	
	MK353129													
Isthmolongispora lanceata	MH858897			S3							S10	S11		S13
Mycoarthris corallina	MN660521			S3							S10		S12	
	MN459707										S10			
Cylindrocladiella elegans	JN943101			S3										
Fusarium avenaceum	MH858036			S3										
Plectosphaerella cucumerina	MK246008													
	MN452657										S10			
Tumularia aquatica	MK371732			S3						S9			S12	S13
	MK371733													
	MK353137			S3						S9	S10		S12	
Curvularia coatesiae	MT341911				S4									
Epicoccum nigrum	MT582797				S4		S6					S11	S12	S13
	MG736195													
	MF435122													
	MT557339											S11		
	MN947593										S10			
	MG602553													
	MK460957													
	MT573480									S9				
	MF509753								S8					
Gyoerffyella rotula	KU516475				S4									
	KU516477													
	KU516473								S8					
Heliscella stellata	MK353113				S4									
	OM907736										S10			S13
Aureobasidium pullulans	MT645930				S4	S5	S6		S8	S9	S10	S11		S13
	MT645923							S7						
Tricladium splendens	MK353136					S5								S13
	MK353134													
Tetracladium breve	MK371730					S5		S7			S10			
	EU883431													
	KC180669													
	FJ000405													
	GQ411301										S10			
Naganishia adeliensis	MT079162						S6							
Alternaria tenuissima	MK798424						S6							
	KF381078													
	MT212230													
Flagellospora fusarioides	MK965839						S6							
Alternaria rosae	MT457663						S6							
Torula pluriseptata	MN061338						S6							
Phoma moricola	MT626622						S6							
Fusarium equiseti	MT558569						S6							
Ascochyta rabiei	MT252615						S6							
Torula herbarum	MN313817						S6							
	MN313818						S6							

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	G	A	A	A	G	A	D	D	A	G
Species	**GenBank**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	**S23**	**S24**	**S25**
Tricladium angulatum	MH930824					S18							
	MK353129						S19						
Isthmolongispora lanceata	MH858897			S16									
Mycoarthris corallina	MN660521												
	MN459707			S16									
Cylindrocladiella elegans	JN943101												
Fusarium avenaceum	MH858036												
Plectosphaerella cucumerina	MK246008												
	MN452657			S16									
Tumularia aquatica	MK371732		S15	S16									
	MK371733			S16									
	MK353137												
Curvularia coatesiae	MT341911												
Epicoccum nigrum	MT582797					S18	S19			S22			
	MG736195				S17								
	MF435122												
	MT557339											S24	
	MN947593		S15										
	MG602553		S15						S21				
	MK460957		S15										
	MT573480								S21				
	MF509753								S21			S24	
Gyoerffyella rotula	KU516475												
	KU516477												
	KU516473												
Heliscella stellata	MK353113												
	OM907736			S16									
Aureobasidium pullulans	MT645930	S14	S15		S17	S18		S20	S21			S24	
	MT645923												
Tricladium splendens	MK353136		S15										
	MK353134			S16									
Tetracladium breve	MK371730										S23		
	EU883431												
	KC180669												
	FJ000405												
	GQ411301												
Naganishia adeliensis	MT079162												
Alternaria tenuissima	MK798424												
	KF381078												
	MT212230												
Flagellospora fusarioides	MK965839												
Alternaria rosae	MT457663												
Torula pluriseptata	MN061338												
Phoma moricola	MT626622												
Fusarium equiseti	MT558569												
Ascochyta rabiei	MT252615												
Torula herbarum	MN313817												
	MN313818												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	C	C	F	F	C	F	F	C	F	C
Species	**GenBank**	**S26**	**S27**	**S28**	**S29**	**S30**	**S31**	**S32**	**S33**	**S34**	**S35**	**S36**	**S37**
Tricladium angulatum	MH930824			S28	S29	S30	S31	S32	S33	S34	S35	S36	S37
	MK353129						S31						
Isthmolongispora lanceata	MH858897					S30	S31		S33				
Mycoarthris corallina	MN660521												
	MN459707											S36	
Cylindrocladiella elegans	JN943101												
Fusarium avenaceum	MH858036												
Plectosphaerella cucumerina	MK246008												
	MN452657	S26											
Tumularia aquatica	MK371732					S30							S37
	MK371733											S36	S37
	MK353137			S28									
Curvularia coatesiae	MT341911										S35		
Epicoccum nigrum	MT582797			S28	S29		S31	S32					S37
	MG736195												
	MF435122												
	MT557339												
	MN947593												
	MG602553						S31				S35		
	MK460957												
	MT573480												
	MF509753												
Gyoerffyella rotula	KU516475												
	KU516477												
	KU516473												
Heliscella stellata	MK353113												
	OM907736			S28	S29								
Aureobasidium pullulans	MT645930		S27	S28			S31	S32	S33		S35	S36	
	MT645923												
Tricladium splendens	MK353136												
	MK353134				S29								
Tetracladium breve	MK371730	S26	S27			S30		S32	S33	S34		S36	S37
	EU883431									S34		S36	
	KC180669							S32				S36	
	FJ000405											S36	
	GQ411301												
Naganishia adeliensis	MT079162												
Alternaria tenuissima	MK798424												
	KF381078												
	MT212230												
Flagellospora fusarioides	MK965839						S31					S36	
Alternaria rosae	MT457663												
Torula pluriseptata	MN061338												
Phoma moricola	MT626622												
Fusarium equiseti	MT558569												
Ascochyta rabiei	MT252615												
Torula herbarum	MN313817												
	MN313818												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	C	F	C	C	F	F	C	F	B	H	H	B
Species	**GenBank**	**S38**	**S39**	**S40**	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S47**	**s48**	**S49**
Tricladium angulatum	MH930824	S38	S39							S46	S47		S49
	MK353129												
Isthmolongispora lanceata	MH858897		S39								S47	S48	
Mycoarthris corallina	MN660521										S47		
	MN459707												
Cylindrocladiella elegans	JN943101												
Fusarium avenaceum	MH858036												
Plectosphaerella cucumerina	MK246008												
	MN452657										S47		
Tumularia aquatica	MK371732										S47		
	MK371733												
	MK353137												
Curvularia coatesiae	MT341911												
Epicoccum nigrum	MT582797	S38	S39	S40	S41	S42		S44	S45		S47		
	MG736195												
	MF435122								S45				
	MT557339												
	MN947593												
	MG602553		S39						S45				
	MK460957												
	MT573480												
	MF509753												
Gyoerffyella rotula	KU516475												
	KU516477												
	KU516473												
Heliscella stellata	MK353113										S47		
	OM907736										S47		
Aureobasidium pullulans	MT645930		S39				S43		S45			S48	
	MT645923												
Tricladium splendens	MK353136	S38											
	MK353134										S47		
Tetracladium breve	MK371730	S38				S42	S43		S45	S46	S47		S49
	EU883431		S39				S43		S45				
	KC180669								S45				
	FJ000405												
	GQ411301												
Naganishia adeliensis	MT079162												
Alternaria tenuissima	MK798424												
	KF381078												
	MT212230							S44					
Flagellospora fusarioides	MK965839										S47		
Alternaria rosae	MT457663							S44					
Torula pluriseptata	MN061338												
Phoma moricola	MT626622												
Fusarium equiseti	MT558569												
Ascochyta rabiei	MT252615												
Torula herbarum	MN313817												
	MN313818												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	E	B	E	E	H	E	B	B	H	B	E	E
Species	**GenBank**	**S50**	**S51**	**S52**	**S53**	**S54**	**S55**	**S56**	**S57**	**S58**	**S59**	**S60**	**S61**
Tricladium angulatum	MH930824	S50	S51	S52	S53		S55	S56	S57		S59		
	MK353129												
Isthmolongispora lanceata	MH858897		S51			S54	S55				S59	S60	
Mycoarthris corallina	MN660521												
	MN459707												
Cylindrocladiella elegans	JN943101												
Fusarium avenaceum	MH858036												
Plectosphaerella cucumerina	MK246008												
	MN452657									S58			
Tumularia aquatica	MK371732								S57		S59		
	MK371733		S51										
	MK353137		S51					S56					
Curvularia coatesiae	MT341911												
Epicoccum nigrum	MT582797		S51	S52					S57	S58		S60	S61
	MG736195												
	MF435122												
	MT557339				S53								S61
	MN947593												
	MG602553										S59	S60	
	MK460957												
	MT573480				S53								S61
	MF509753										S59	S60	
Gyoerffyella rotula	KU516475												
	KU516477		S51										
	KU516473												
Heliscella stellata	MK353113			S52									
	OM907736										S59		
Aureobasidium pullulans	MT645930	S50		S52	S53		S55		S57		S59	S60	S61
	MT645923												
Tricladium splendens	MK353136												
	MK353134												
Tetracladium breve	MK371730	S50	S51						S57		S59	S60	S61
	EU883431												
	KC180669												S61
	FJ000405												
	GQ411301												
Naganishia adeliensis	MT079162												
Alternaria tenuissima	MK798424												
	KF381078				S53								
	MT212230												
Flagellospora fusarioides	MK965839												
Alternaria rosae	MT457663												
Torula pluriseptata	MN061338												
Phoma moricola	MT626622												
Fusarium equiseti	MT558569												
Ascochyta rabiei	MT252615												
Torula herbarum	MN313817												
	MN313818												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	H	H	E	B	B	B	E	H	H	E	H
Species	**GenBank**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Tricladium angulatum	MH930824											
	MK353129											
Isthmolongispora lanceata	MH858897	S62										
Mycoarthris corallina	MN660521							S68				
	MN459707											
Cylindrocladiella elegans	JN943101											
Fusarium avenaceum	MH858036											
Plectosphaerella cucumerina	MK246008											
	MN452657	S62										
Tumularia aquatica	MK371732											
	MK371733											
	MK353137											
Curvularia coatesiae	MT341911											
Epicoccum nigrum	MT582797			S64								
	MG736195											
	MF435122											
	MT557339						S67					
	MN947593											
	MG602553											
	MK460957											
	MT573480											
	MF509753											
Gyoerffyella rotula	KU516475											
	KU516477											
	KU516473											
Heliscella stellata	MK353113											
	OM907736	S62	S63									
Aureobasidium pullulans	MT645930	S62	S63	S64					S69			
	MT645923											
Tricladium splendens	MK353136											
	MK353134											
Tetracladium breve	MK371730	S62		S64				S68		S70		
	EU883431											
	KC180669											
	FJ000405											
	GQ411301											
Naganishia adeliensis	MT079162											
Alternaria tenuissima	MK798424											
	KF381078											
	MT212230											
Flagellospora fusarioides	MK965839	S62										
Alternaria rosae	MT457663											
Torula pluriseptata	MN061338											
Phoma moricola	MT626622											
Fusarium equiseti	MT558569											
Ascochyta rabiei	MT252615											
Torula herbarum	MN313817											
	MN313818											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	A	G	A	G	A	D	D	D	G	D	D	S
Species	**GenBank**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**
Torula acaciae	NR_155944						S6							
Alternaria alternata	ON208172						S6							
	MN615420													
	MT646481													
	MW741555						S6							
Flagellospora leucorhynchos	KC834049						S6				S10			S13
Tumularia tuberculata	MK371734						S6							S13
Taphrina sadebeckii	AY090488							S7						
	NR_155882													
Pseudocoleophoma polygonicola	MZ492974									S9				***P***
Boeremia exigua var. exigua	MT397284									S9				
	MN540289													
Xenodidymella applanata	MT573496									S9				
Arxiella terrestris	MH858565										S10		S12	
Plectosphaerella plurivora	MN249563										S10			
Apiotrichum porosum	MT502794										S10			
Neonectria lugdunensis	MK803117										S10			
	MK353115													
Alatospora pulchella	MZ773536										S10		S12	
	MN660457													
	KC834039													
	KF730800													
	KF730803												S12	
Trichocladium acropullum	MH864229										S10			
Leptodontidium trabinellum	KY853449										S10			
Dactylonectria macrodidyma	MK841907										S10			
Filobasidium globisporum	LC515032											S11		
Lemonniera aquatica	MK226460											S11		
	OM907740													
	MK353145												S12	
Dactylonectria torresensis	MN988721												S12	
Vargamyces aquaticus	KF280586												S12	
	MZ492962													
Kalmusia variispora	MG208005												S12	
Hymenoscyphus cf. imberbis	OL679974												S12	
Neopyrenochaeta annellidica	LR897774												S12	
	MT185538													
Lophiostoma rugulosum	NR_160228													S13
Psychrophila olivacea	JX001622													S13
Fusarium acuminatum	MT566456													
	MT635295													
Neopyrenochaeta maesuayensis	LR897776													
	NR_170043													
	LR897782													
	MT185540													
Pyrenochaetopsis leptospora	MT453283													
Neodidymelliopsis cannabis	MH859057													

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	G	A	A	A	G	A	D	D	A	G
Species	**GenBank**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	**S23**	**S24**	**S25**
Torula acaciae	NR_155944												
Alternaria alternata	ON208172												
	MN615420												
	MT646481												
	MW741555												
Flagellospora leucorhynchos	KC834049												
Tumularia tuberculata	MK371734												
Taphrina sadebeckii	AY090488					S18							
	NR_155882												
eudocoleophoma polygonico	MZ492974												
Boeremia exigua var. exigua	MT397284												
	MN540289												
Xenodidymella applanata	MT573496												
Arxiella terrestris	MH858565												S25
Plectosphaerella plurivora	MN249563												
Apiotrichum porosum	MT502794							S20					
Neonectria lugdunensis	MK803117							S20	S21				
	MK353115												
Alatospora pulchella	MZ773536			S16									
	MN660457			S16									
	KC834039												
	KF730800												
	KF730803			S16									
Trichocladium acropullum	MH864229												
Leptodontidium trabinellum	KY853449												
Dactylonectria macrodidyma	MK841907		S15	S16							S23		
Filobasidium globisporum	LC515032												
Lemonniera aquatica	MK226460												
	OM907740												
	MK353145												
Dactylonectria torresensis	MN988721												
Vargamyces aquaticus	KF280586			S16									
	MZ492962			S16									
Kalmusia variispora	MG208005												
Hymenoscyphus cf. imberbis	OL679974			S16									
Neopyrenochaeta annellidica	LR897774												
	MT185538	S14											
Lophiostoma rugulosum	NR_160228												
Psychrophila olivacea	JX001622												
Fusarium acuminatum	MT566456	S14											
	MT635295									S22			
eopyrenochaeta maesuayens	LR897776	S14											
	NR_170043												
	LR897782												
	MT185540	S14											
Pyrenochaetopsis leptospora	MT453283	S14											
Neodidymelliopsis cannabis	MH859057		S15										

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	C	C	F	F	C	F	F	C	F	C
Species	**GenBank**	**S26**	**S27**	**S28**	**S29**	**S30**	**S31**	**S32**	**S33**	**S34**	**S35**	**S36**	**S37**
Torula acaciae	NR_155944												
Alternaria alternata	ON208172												
	MN615420												
	MT646481												
	MW741555										S35		
Flagellospora leucorhynchos	KC834049			S28	S29	S30	S31	S32		S34		S36	S37
Tumularia tuberculata	MK371734						S31						
Taphrina sadebeckii	AY090488		S27										
	NR_155882						S31		S33				
seudocoleophoma polygonicol	MZ492974												***P***
Boeremia exigua var. exigua	MT397284												
	MN540289												
Xenodidymella applanata	MT573496												
Arxiella terrestris	MH858565	S26				S30							
Plectosphaerella plurivora	MN249563												
Apiotrichum porosum	MT502794							S32					
Neonectria lugdunensis	MK803117			S28									
	MK353115												
Alatospora pulchella	MZ773536			S28	S29	S30		S32		S34		S36	S37
	MN660457												
	KC834039					S30							
	KF730800						S31			S34			
	KF730803					S30	S31					S36	
Trichocladium acropullum	MH864229												
Leptodontidium trabinellum	KY853449												
Dactylonectria macrodidyma	MK841907											S36	
Filobasidium globisporum	LC515032							S32					
Lemonniera aquatica	MK226460												
	OM907740		S27										
	MK353145					S30						S36	
Dactylonectria torresensis	MN988721												
Vargamyces aquaticus	KF280586					S30			S33			S36	
	MZ492962					S30			S33				
Kalmusia variispora	MG208005												
Hymenoscyphus cf. imberbis	OL679974												
Neopyrenochaeta annellidica	LR897774							S32				S36	
	MT185538												
Lophiostoma rugulosum	NR_160228												
Psychrophila olivacea	JX001622					S30							
Fusarium acuminatum	MT566456												
	MT635295												
Neopyrenochaeta maesuayensi	LR897776												
	NR_170043												
	LR897782												
	MT185540												
Pyrenochaetopsis leptospora	MT453283												
Neodidymelliopsis cannabis	MH859057												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	C	F	C	C	F	F	C	F	B	H	H	B
Species	**GenBank**	**S38**	**S39**	**S40**	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S47**	**s48**	**S49**
Torula acaciae	NR_155944												
Alternaria alternata	ON208172												
	MN615420				S41			S44					
	MT646481			S40				S44					
	MW741555												
Flagellospora leucorhynchos	KC834049			S40						S46			
Tumularia tuberculata	MK371734									S46			
Taphrina sadebeckii	AY090488								S45				
	NR_155882												
seudocoleophoma polygonicol	MZ492974												***P***
Boeremia exigua var. exigua	MT397284												
	MN540289												
Xenodidymella applanata	MT573496												
Arxiella terrestris	MH858565												
Plectosphaerella plurivora	MN249563												
Apiotrichum porosum	MT502794												
Neonectria lugdunensis	MK803117			S40									
	MK353115			S40									
Alatospora pulchella	MZ773536										S47		
	MN660457												
	KC834039												
	KF730800	S38								S46			
	KF730803		S39										
Trichocladium acropullum	MH864229												
Leptodontidium trabinellum	KY853449										S47		
Dactylonectria macrodidyma	MK841907			S40		S42			S45				
Filobasidium globisporum	LC515032												
Lemonniera aquatica	MK226460												
	OM907740												
	MK353145												
Dactylonectria torresensis	MN988721				S41								
Vargamyces aquaticus	KF280586			S40									
	MZ492962										S47		
Kalmusia variispora	MG208005												
Hymenoscyphus cf. imberbis	OL679974												
Neopyrenochaeta annellidica	LR897774						S43						
	MT185538									S46			
Lophiostoma rugulosum	NR_160228												
Psychrophila olivacea	JX001622												
Fusarium acuminatum	MT566456												
	MT635295								S45				
Neopyrenochaeta maesuayensi	LR897776												
	NR_170043												
	LR897782												
	MT185540											S48	
Pyrenochaetopsis leptospora	MT453283												
Neodidymelliopsis cannabis	MH859057												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	E	B	E	E	H	E	B	B	H	B	E	E
Species	**GenBank**	**S50**	**S51**	**S52**	**S53**	**S54**	**S55**	**S56**	**S57**	**S58**	**S59**	**S60**	**S61**
Torula acaciae	NR_155944												
Alternaria alternata	ON208172												
	MN615420												
	MT646481											S60	
	MW741555												
Flagellospora leucorhynchos	KC834049		S51				S55	S56			S59		
Tumularia tuberculata	MK371734												
Taphrina sadebeckii	AY090488				S53								
	NR_155882												S61
:eudocoleophoma polygonico	MZ492974												***P***
Boeremia exigua var. exigua	MT397284												
	MN540289				S53								
Xenodidymella applanata	MT573496												
Arxiella terrestris	MH858565												
Plectosphaerella plurivora	MN249563												S61
Apiotrichum porosum	MT502794										S59		
Neonectria lugdunensis	MK803117												
	MK353115												
Alatospora pulchella	MZ773536		S51		S53			S56	S57	S58	S59	S60	
	MN660457												
	KC834039												
	KF730800												
	KF730803										SS59		
Trichocladium acropullum	MH864229												
Leptodontidium trabinellum	KY853449												
Dactylonectria macrodidyma	MK841907		S51			S54					S59		
Filobasidium globisporum	LC515032				S53								
Lemonniera aquatica	MK226460						S55						
	OM907740												
	MK353145		S51					S56			S59	S60	S61
Dactylonectria torresensis	MN988721							S56					
Vargamyces aquaticus	KF280586		S51		S53		S55	S56				S60	S61
	MZ492962												
Kalmusia variispora	MG208005												
Hymenoscyphus cf. imberbis	OL679974												
Neopyrenochaeta annellidica	LR897774	S50		S52	S53	S54	S55				S59	S60	S61
	MT185538			S52	S53	S54		S56		S58		S60	S61
Lophiostoma rugulosum	NR_160228												
Psychrophila olivacea	JX001622												
Fusarium acuminatum	MT566456												
	MT635295												
eopyrenochaeta maesuayens	LR897776												
	NR_170043							S56					
	LR897782				S53								
	MT185540					S54		S56		S58			S61
Pyrenochaetopsis leptospora	MT453283												
Neodidymelliopsis cannabis	MH859057												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	H	H	E	B	B	B	E	H	H	E	H
Species	**GenBank**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Torula acaciae	NR_155944											
Alternaria alternata	ON208172											
	MN615420											
	MT646481											
	MW741555											
Flagellospora leucorhynchos	KC834049	S62										
Tumularia tuberculata	MK371734											
Taphrina sadebeckii	AY090488											
	NR_155882								S69			
seudocoleophoma polygonicol	MZ492974											
Boeremia exigua var. exigua	MT397284											
	MN540289											
Xenodidymella applanata	MT573496											
Arxiella terrestris	MH858565						S67					
Plectosphaerella plurivora	MN249563	S62										
Apiotrichum porosum	MT502794									S70		
Neonectria lugdunensis	MK803117											
	MK353115											
Alatospora pulchella	MZ773536	S62										
	MN660457											
	KC834039											
	KF730800											
	KF730803	S62										
Trichocladium acropullum	MH864229											
Leptodontidium trabinellum	KY853449											
Dactylonectria macrodidyma	MK841907						S67		S69			
Filobasidium globisporum	LC515032											
Lemonniera aquatica	MK226460											
	OM907740											
	MK353145											
Dactylonectria torresensis	MN988721											
Vargamyces aquaticus	KF280586											
	MZ492962											
Kalmusia variispora	MG208005											
Hymenoscyphus cf. imberbis	OL679974											
Neopyrenochaeta annellidica	LR897774											
	MT185538											
Lophiostoma rugulosum	NR_160228											
Psychrophila olivacea	JX001622											
Fusarium acuminatum	MT566456											
	MT635295											
leopyrenochaeta maesuayensi	LR897776											
	NR_170043											
	LR897782											
	MT185540											
Pyrenochaetopsis leptospora	MT453283		S63									
Neodidymelliopsis cannabis	MH859057											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	G	A	A	A	G	A	D	D	A	G
Species	**GenBank**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	**S23**	**S24**	**S25**
Boeremia galiicola	MT177919		S15		S17								
Tricellula inaequalis	MH857245		S15										
Paraconiothyrium fuckelii	MK911699			S16									
Cosmosporella olivacea	MH087212			S16									
Clohesyomyces aquaticus	MF110612			S16									
Cadophora luteo-olivacea	MK919500			S16									
	MN232940												
Camposporium multiseptatun	NR_171863			S16									
Anguillospora crassa	MK371722			S16									
Dimorphospora foliicola	MZ773538			S16				S20					
Microsphaeropsis olivacea	MH871969				S17								
Alternaria abundans	MH861640				S17								
Epicoccum huancayense	MN077427				S17								
Helicodendron articulatum	MH856857				S17								
Helicodendron triglitziense	MK432688				S17								
Didymella pinodella	MT555747						S19						
Alternaria infectoria	MT561399							S20					
Pythium aff. attrantheridum	MN306101							S20					
Paramyrothecium roridum	KU529828									S22			
Alternaria longipes	LC269927									S22			
	MT635195												
Didymella prosopidis	MT605129										S23		
Phyllactinia betulae	ON073889												
Cylindrodendrum hubeiense	MT151680												
	KR816357												
Kondoa phyllada	KY103886												
Coprinellus micaceus	MT644910												
Towyspora aestuari	NR_148095												
Helicodendron luteoalbum	MK965755												
Geniculospora inflata	OM907735												
Septoriella oudemansii	MN966618												
Tetracladium setigerum	EU883425												
	EU883427												
	EU883426												
Triangularia longicaudata	KT224794												
Tetracladium furcatum	MK353120												
Tetracladium maxilliforme	KU519119												
	MK353128												
Ilyonectria robusta	MN817711												
	MN450583												
Amniculicola lignicola	OM337526												
Fusarium reticulatum	MT601889												
Taphrina alni	AF492076												
	AF492077												
Articulospora proliferata	KP234351												
oleophoma paracylindrospor	KU728492												
Periconia macrospinosa	MK841459												
Tausonia pullulans	KY646441												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	G	D	C	C	F	F	C	F	F	C	F	C
Species	**GenBank**	**S26**	**S27**	**S28**	**S29**	**S30**	**S31**	**S32**	**S33**	**S34**	**S35**	**S36**	**S37**
Boeremia galiicola	MT177919							S32	S33				
Tricellula inaequalis	MH857245												
Paraconiothyrium fuckelii	MK911699												
Cosmosporella olivacea	MH087212												
Clohesyomyces aquaticus	MF110612												
Cadophora luteo-olivacea	MK919500												
	MN232940												
Camposporium multiseptatum	NR_171863												
Anguillospora crassa	MK371722												
Dimorphospora foliicola	MZ773538				S29								
Microsphaeropsis olivacea	MH871969												
Alternaria abundans	MH861640										S35		
Epicoccum huancayense	MN077427												
Helicodendron articulatum	MH856857												
Helicodendron triglitziense	MK432688												
Didymella pinodella	MT555747												
Alternaria infectoria	MT561399												
Pythium aff. attrantheridum	MN306101												
Paramyrothecium roridum	KU529828												
Alternaria longipes	LC269927										S35		
	MT635195												
Didymella prosopidis	MT605129												
Phyllactinia betulae	ON073889	S26											
Cylindrodendrum hubeiense	MT151680				S29								
	KR816357									S34			
Kondoa phyllada	KY103886						S31						
Coprinellus micaceus	MT644910							S32					
Towyspora aestuari	NR_148095							S32					
Helicodendron luteoalbum	MK965755							S32					
Geniculospora inflata	OM907735							S32			S35		
Septoriella oudemansii	MN966618								S33				
Tetracladium setigerum	EU883425								S33			S36	
	EU883427												
	EU883426												
Triangularia longicaudata	KT224794								S33				
Tetracladium furcatum	MK353120											S36	
Tetracladium maxilliforme	KU519119											S36	
	MK353128												
Ilyonectria robusta	MN817711											S36	
	MN450583												
Amniculicola lignicola	OM337526											S36	
Fusarium reticulatum	MT601889												S37
Taphrina alni	AF492076												
	AF492077												
Articulospora proliferata	KP234351												
Coleophoma paracylindrospora	KU728492												
Periconia macrospinosa	MK841459												
Tausonia pullulans	KY646441												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	C	F	C	C	F	F	C	F	B	H	H	B
Species	**GenBank**	**S38**	**S39**	**S40**	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S47**	**s48**	**S49**
Boeremia galiicola	MT177919												
Tricellula inaequalis	MH857245												
Paraconiothyrium fuckelii	MK911699												
Cosmosporella olivacea	MH087212												
Clohesyomyces aquaticus	MF110612												
Cadophora luteo-olivacea	MK919500												
	MN232940	S38											
Camposporium multiseptatum	NR_171863												
Anguillospora crassa	MK371722												
Dimorphospora foliicola	MZ773538	S38							S45				
Microsphaeropsis olivacea	MH871969												
Alternaria abundans	MH861640												
Epicoccum huancayense	MN077427												
Helicodendron articulatum	MH856857												
Helicodendron triglitziense	MK432688										S47		
Didymella pinodella	MT555747												
Alternaria infectoria	MT561399						S43						
Pythium aff. attrantheridum	MN306101												
Paramyrothecium roridum	KU529828												
Alternaria longipes	LC269927												
	MT635195												
Didymella prosopidis	MT605129												
Phyllactinia betulae	ON073889												
Cylindrodendrum hubeiense	MT151680												
	KR816357												
Kondoa phyllada	KY103886												
Coprinellus micaceus	MT644910										S47		
Towyspora aestuari	NR_148095												
Helicodendron luteoalbum	MK965755												
Geniculospora inflata	OM907735	S38											
Septoriella oudemansii	MN966618												
Tetracladium setigerum	EU883425	S38					S43		S45				
	EU883427									S46			
	EU883426						S43						
Triangularia longicaudata	KT224794												
Tetracladium furcatum	MK353120						S43						
Tetracladium maxilliforme	KU519119												
	MK353128	S38											
Ilyonectria robusta	MN817711												
	MN450583				S41								
Amniculicola lignicola	OM337526												
Fusarium reticulatum	MT601889												
Taphrina alni	AF492076	S38											
	AF492077												
Articulospora proliferata	KP234351	S38											
Coleophoma paracylindrospora	KU728492	S38											
Periconia macrospinosa	MK841459		S39										
Tausonia pullulans	KY646441	S38											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	E	B	E	E	H	E	B	B	H	B	E	E
Species	**GenBank**	**S50**	**S51**	**S52**	**S53**	**S54**	**S55**	**S56**	**S57**	**S58**	**S59**	**S60**	**S61**
Boeremia galiicola	MT177919		S51										
Tricellula inaequalis	MH857245												
Paraconiothyrium fuckelii	MK911699												
Cosmosporella olivacea	MH087212												
Clohesyomyces aquaticus	MF110612												
Cadophora luteo-olivacea	MK919500												
	MN232940												
Camposporium multiseptatum	NR_171863												
Anguillospora crassa	MK371722												
Dimorphospora foliicola	MZ773538												
Microsphaeropsis olivacea	MH871969												
Alternaria abundans	MH861640												
Epicoccum huancayense	MN077427												
Helicodendron articulatum	MH856857												
Helicodendron triglitziense	MK432688												
Didymella pinodella	MT555747												
Alternaria infectoria	MT561399												
Pythium aff. attrantheridum	MN306101												
Paramyrothecium roridum	KU529828												
Alternaria longipes	LC269927												
	MT635195								S57				
Didymella prosopidis	MT605129												
Phyllactinia betulae	ON073889												
Cylindrodendrum hubeiense	MT151680												
	KR816357												
Kondoa phyllada	KY103886												
Coprinellus micaceus	MT644910			S52		S54							
Towyspora aestuari	NR_148095												
Helicodendron luteoalbum	MK965755												
Geniculospora inflata	OM907735												
Septoriella oudemansii	MN966618			S52									
Tetracladium setigerum	EU883425								S57				
	EU883427												
	EU883426												
Triangularia longicaudata	KT224794												
Tetracladium furcatum	MK353120												
Tetracladium maxilliforme	KU519119												
	MK353128												
Ilyonectria robusta	MN817711												
	MN450583												
Amniculicola lignicola	OM337526							S56					
Fusarium reticulatum	MT601889												
Taphrina alni	AF492076												
	AF492077												
Articulospora proliferata	KP234351												
Coleophoma paracylindrospor	KU728492												
Periconia macrospinosa	MK841459												
Tausonia pullulans	KY646441												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	H	H	E	B	B	B	E	H	H	E	H
Species	**GenBank**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Boeremia galiicola	MT177919	S62										
Tricellula inaequalis	MH857245											
Paraconiothyrium fuckelii	MK911699											
Cosmosporella olivacea	MH087212											
Clohesyomyces aquaticus	MF110612											
Cadophora luteo-olivacea	MK919500											
	MN232940											
Camposporium multiseptatum	NR_171863											
Anguillospora crassa	MK371722											
Dimorphospora foliicola	MZ773538		S63									
Microsphaeropsis olivacea	MH871969											
Alternaria abundans	MH861640											
Epicoccum huancayense	MN077427											
Helicodendron articulatum	MH856857											
Helicodendron triglitziense	MK432688											
Didymella pinodella	MT555747											
Alternaria infectoria	MT561399											
Pythium aff. attrantheridum	MN306101											
Paramyrothecium roridum	KU529828											
Alternaria longipes	LC269927											
	MT635195											
Didymella prosopidis	MT605129											
Phyllactinia betulae	ON073889											
Cylindrodendrum hubeiense	MT151680											
	KR816357						S67					
Kondoa phyllada	KY103886											
Coprinellus micaceus	MT644910											
Towyspora aestuari	NR_148095											
Helicodendron luteoalbum	MK965755											
Geniculospora inflata	OM907735											
Septoriella oudemansii	MN966618											
Tetracladium setigerum	EU883425	S62										
	EU883427											
	EU883426											
Triangularia longicaudata	KT224794											
Tetracladium furcatum	MK353120											
Tetracladium maxilliforme	KU519119											
	MK353128											
Ilyonectria robusta	MN817711									S70		
	MN450583											
Amniculicola lignicola	OM337526											
Fusarium reticulatum	MT601889											
Taphrina alni	AF492076											
	AF492077				S65							
Articulospora proliferata	KP234351											
Coleophoma paracylindrospora	KU728492											
Periconia macrospinosa	MK841459											
Tausonia pullulans	KY646441											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	C	F	C	C	F	F	C	F	B	H	H	B
Species	**GenBank**	**S38**	**S39**	**S40**	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S47**	**s48**	**S49**
Cylindrodendrum alicantinum	NR_158396			S40									
	KX610385					S42							
Paraphoma chrysanthemicola	MK647980			S40									S49
Dactylonectria estremocensis	LR875330				S41								
Phoma herbarum	KJ191690					s42							
Truncatella angustata	MT514378						S43						
Letendraea helminthicola	MK389410							S44					
Chaetopyrena penicillata	MK100129							S44					
Curvularia inaequalis	MT229249									S46			
Coprinellus disseminatus	MK801349										S47		
Knufia perfecta	MF062036										S47		
Orbilia xinjiangensis	MH856835										S47		
Vishniacozyma victoriae	MK782476										S47		
	KX067806												
Myrmecridium schulzeri	MT446214											S48	
Campylospora chaetocladia	JN190876												S49
Arthrobotrys xiangyunensis	KT215214												S49
Neopyrenochaeta acicola	NR_160055												S49
	KJ395501												
Ascochyta herbicola	MN660400												
Sterkiella nova	AF508771												
Sterkiella histriomuscorum	FJ545743												
agonosporopsis cucurbitacearu	MK690410												***St***
Dendryphion nanum	MN999921												
Coprinopsis marcescibilis	MH856262												
Tympanis malicola	MK314579												
Aspergillus penicillioides	HQ891824												
Lentithecium aquaticum	NR_160229												
Alternaria brassicae	KF543046												
Uzbekistanica yakutkhanika	NR_157550												
Neopyrenochaeta telephoni	MK005257												
	KM516291												
Cylindrocladiella pseudoparva	NR_111650												
Saccothecium rubi	MH627280												
Alternaria triticina	MN313292												
Hannaella luteola	MK998685												
Plectosphaerella oligotrophica	MT447499												
Fusarium merismoides	MK397278												
Filobasidium magnum	MT635292												
Volutella ciliata	MH892587												
Bjerkandera adusta	MH237826												
Tulosesus callinus	MH856992												
Hypholoma fasciculare	MK050598												
Fusicolla acetilerea	MG256500												
Alloleptosphaeria iridicola	NR_159068												
Didymella musae	MN686292												
Kirschsteiniothelia arasbaranica	KX621986												***K***
Dendryphion comosum	MH859293												

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	E	B	E	E	H	E	B	B	H	B	E	E
Species	**GenBank**	**S50**	**S51**	**S52**	**S53**	**S54**	**S55**	**S56**	**S57**	**S58**	**S59**	**S60**	**S61**
Cylindrodendrum alicantinum	NR_158396							S56					
	KX610385												
Paraphoma chrysanthemicola	MK647980				S53						S59		
Dactylonectria estremocensis	LR875330												
Phoma herbarum	KJ191690												
Truncatella angustata	MT514378												
Letendraea helminthicola	MK389410												
Chaetopyrena penicillata	MK100129												
Curvularia inaequalis	MT229249												
Coprinellus disseminatus	MK801349												
Knufia perfecta	MF062036												
Orbilia xinjiangensis	MH856835												
Vishniacozyma victoriae	MK782476												
	KX067806											S60	
Myrmecridium schulzeri	MT446214												
Campylospora chaetocladia	JN190876												
Arthrobotrys xiangyunensis	KT215214												
Neopyrenochaeta acicola	NR_160055												
	KJ395501									S58			
Ascochyta herbicola	MN660400	S50											
Sterkiella nova	AF508771			S52									S61
Sterkiella histriomuscorum	FJ545743			S52									S61
gonosporopsis cucurbitacear	MK690410			S52									***St***
Dendryphion nanum	MN999921			S52	S53		S55						
Coprinopsis marcescibilis	MH856262			S52									
Tympanis malicola	MK314579					S54							
Aspergillus penicillioides	HQ891824												
Lentithecium aquaticum	NR_160229						S55						
Alternaria brassicae	KF543046						S55						
Uzbekistanica yakutkhanika	NR_157550							S56					
Neopyrenochaeta telephoni	MK005257							S56					
	KM516291											S60	
Cylindrocladiella pseudoparva	NR_111650							S56					
Saccothecium rubi	MH627280								S57				
Alternaria triticina	MN313292								S57				
Hannaella luteola	MK998685								S57				
Plectosphaerella oligotrophica	MT447499									S58			
Fusarium merismoides	MK397278										S59		
Filobasidium magnum	MT635292											S60	
Volutella ciliata	MH892587											S60	
Bjerkandera adusta	MH237826											S60	
Tulosesus callinus	MH856992												S61
Hypholoma fasciculare	MK050598												S61
Fusicolla acetilerea	MG256500												S61
Alloleptosphaeria iridicola	NR_159068												S61
Didymella musae	MN686292												S61
Irschsteiniothelia arasbaranica	KX621986												S61
Dendryphion comosum	MH859293												S61

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	H	H	E	B	B	B	E	H	H	E	H
Species	**GenBank**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Cylindrodendrum alicantinum	NR_158396											
	KX610385											
Paraphoma chrysanthemicola	MK647980											
Dactylonectria estremocensis	LR875330											
Phoma herbarum	KJ191690											
Truncatella angustata	MT514378											
Letendraea helminthicola	MK389410											
Chaetopyrena penicillata	MK100129											
Curvularia inaequalis	MT229249											
Coprinellus disseminatus	MK801349											
Knufia perfecta	MF062036											
Orbilia xinjiangensis	MH856835											
Vishniacozyma victoriae	MK782476											
	KX067806											
Myrmecridium schulzeri	MT446214											
Campylospora chaetocladia	JN190876											
Arthrobotrys xiangyunensis	KT215214											
Neopyrenochaeta acicola	NR_160055											S72
	KJ395501											
Ascochyta herbicola	MN660400											
Sterkiella nova	AF508771							S68				
Sterkiella histriomuscorum	FJ545743											
gonosporopsis cucurbitacear	MK690410											
Dendryphion nanum	MN999921											
Coprinopsis marcescibilis	MH856262											
Tympanis malicola	MK314579											
Aspergillus penicillioides	HQ891824											
Lentithecium aquaticum	NR_160229											
Alternaria brassicae	KF543046									S70		
Uzbekistanica yakutkhanika	NR_157550											
Neopyrenochaeta telephoni	MK005257											
	KM516291											
Cylindrocladiella pseudoparva	NR_111650											
Saccothecium rubi	MH627280											
Alternaria triticina	MN313292										S71	
Hannaella luteola	MK998685											
Plectosphaerella oligotrophica	MT447499											
Fusarium merismoides	MK397278											
Filobasidium magnum	MT635292											
Volutella ciliata	MH892587											
Bjerkandera adusta	MH237826											
Tulosesus callinus	MH856992											
Hypholoma fasciculare	MK050598											
Fusicolla acetilerea	MG256500											
Alloleptosphaeria iridicola	NR_159068											
Didymella musae	MN686292											
irschsteiniothelia arasbaranic	KX621986											
Dendryphion comosum	MH859293											

Table S6 – Species name curation from blast (Genbank), including genbank accession number- continuation.

	Site	H	H	E	B	B	B	E	H	H	E	H
Species	**GenBank**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Dendryphion europaeum	NR_158390											
Hyaloscypha spinulosa	MK432695	S62										
Volutella rosea	MH864864	S62										
Phallus impudicus	MT512648		S63									
Clathrus archeri	KP688381		S63									
tagonosporopsis stuijvenberg	MN823449				S65							
Pichia kluyveri	MN268784						S67					
Cladosporium halotolerans	MT626047						S67					
Saccharomyces bayanus	MK267707							S68				
Fusarium oxysporum	MT482502							S68				S72
Preussia minima	MN341252								S69			
Tetracladium apiense	OK037615								S69			
Phomatodes nebulosa	MK100155										S71	
Acremonium fusidioides	HF680224											S72

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments.

SAMPLE	Treat	Nutri	History	Site	*Tetracladium marchalianum*	*Clavariopsis aquatica*	*Alatospora acuminata*	*Amniculicola guttulata*	*Margaritispora aquatica*	*Flagellospora curvula*
S1	0	LOW	P	G	1	1	1	1	1	0
S2	0	VLOW	P	A	1	1	1	1	1	1
S3	0	HIGH	P	G	1	1	1	1	0	1
S4	0	LOW	P	A	0	1	1	0	0	0
S5	0	VLOW	P	G	1	1	1	0	0	1
S6	0	HIGH	P	A	0	1	1	0	0	1
S7	0	VLOW	P	D	1	1	1	1	0	1
S8	0	LOW	P	D	1	1	1	1	1	1
S9	0	HIGH	P	D	0	1	1	1	0	1
S10	30	HIGH	P	G	1	1	1	1	1	0
S11	30	LOW	P	D	0	1	1	1	0	1
S12	30	VLOW	P	D	1	1	1	1	0	0
S13	30	HIGH	P	S	0	1	1	1	0	1
S14	30	LOW	P	G	1	1	1	1	1	0
S15	30	HIGH	P	D	1	1	1	1	1	1
S16	30	VLOW	P	G	1	1	1	1	0	1
S17	30	LOW	P	A	0	1	1	0	1	0
S18	30	VLOW	P	A	0	1	1	0	0	1
S19	300	HIGH	P	A	0	0	1	0	1	0
S20	300	VLOW	P	G	1	0	1	0	1	1
S21	300	VLOW	P	A	1	0	0	0	1	0
S22	300	HIGH	P	D	1	1	1	0	1	1
S23	300	LOW	P	D	1	0	1	0	0	0
S24	300	LOW	P	A	0	0	0	0	0	0
S25	300	HIGH	P	G	1	0	0	0	1	0
S26	300	LOW	P	G	1	1	0	0	1	0
S27	300	VLOW	P	D	1	1	0	1	0	1
S28	0	HIGH	V	C	1	1	1	1	1	1
S29	0	LOW	V	C	1	1	1	1	1	0
S30	0	HIGH	V	F	1	1	1	1	0	0
S31	0	LOW	V	F	1	1	1	1	1	1
S32	0	VLOW	V	C	1	1	1	1	1	1
S33	0	VLOW	V	F	1	1	1	1	1	1
S34	30	HIGH	V	F	1	1	1	1	1	0
S35	30	VLOW	V	C	1	0	1	0	0	1
S36	30	LOW	V	F	1	1	1	1	1	0
S37	30	HIGH	V	C	1	1	1	1	1	0
S38	30	LOW	V	C	1	1	1	1	1	1
S39	30	VLOW	V	F	1	1	1	1	0	1
S40	300	HIGH	V	C	1	0	0	0	1	0
S41	300	LOW	V	C	1	0	0	1	1	0
S42	300	HIGH	V	F	1	0	1	0	1	1
S43	300	LOW	V	F	1	0	0	0	1	0
S44	300	VLOW	V	C	1	0	0	0	0	0
S45	300	VLOW	V	F	1	1	0	0	1	1
S46	0	HIGH	W	B	1	1	1	1	1	1
S47	0	LOW	W	H	1	1	1	1	1	1
S48	0	VLOW	W	H	0	1	0	1	1	1
S49	0	VLOW	W	B	1	1	1	1	0	1
S50	0	VLOW	W	E	1	1	0	1	1	0
S51	0	LOW	W	B	1	1	1	1	1	1
S52	0	HIGH	W	E	1	1	0	1	1	0
S53	0	LOW	W	E	1	1	1	1	0	0
S54	0	HIGH	W	H	1	1	0	1	0	0
S55	30	VLOW	W	E	1	1	0	1	1	1

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Aureobasidium pullulans*	*Epicoccum nigrum*	*Amniculicola longissima*	*Pithomyces chartarum*	*Clavatospora longibrachiata*	*Pseudopithomyces palmicola*	*Stenocladiella neglecta*	*Lemonniera terrestris*
S1	0	0	0	0	1	0	1	0
S2	0	0	1	1	0	1	1	1
S3	0	0	1	0	1	0	1	0
S4	1	1	0	1	0	1	1	1
S5	1	0	1	0	1	1	1	0
S6	1	1	0	1	0	0	1	1
S7	1	0	0	0	0	1	0	1
S8	1	1	0	0	1	0	1	1
S9	1	1	0	1	0	1	1	1
S10	1	1	1	0	1	0	1	0
S11	1	1	0	1	1	1	1	1
S12	0	1	1	1	1	0	0	0
S13	1	1	0	0	1	1	1	1
S14	1	0	0	0	1	0	1	0
S15	1	1	0	1	1	1	1	0
S16	0	0	1	0	1	0	1	1
S17	1	1	1	1	0	1	0	1
S18	1	1	1	1	0	1	0	1
S19	0	1	0	0	0	0	0	0
S20	1	0	0	0	0	0	0	0
S21	1	1	0	1	0	1	0	0
S22	0	1	0	0	0	1	0	0
S23	0	0	0	1	0	0	0	1
S24	1	1	1	1	0	1	0	1
S25	0	0	1	0	1	0	0	0
S26	0	0	1	0	1	0	0	0
S27	1	0	0	0	0	1	0	1
S28	1	1	0	1	1	1	1	1
S29	0	1	1	1	0	1	1	1
S30	0	0	1	1	1	0	0	0
S31	1	1	1	1	0	1	1	0
S32	1	1	1	1	1	1	0	1
S33	1	0	1	0	1	1	1	0
S34	0	0	1	0	0	0	1	0
S35	1	1	1	1	0	1	0	1
S36	1	0	1	1	1	0	0	1
S37	0	1	1	1	0	1	0	1
S38	0	1	0	1	0	1	1	1
S39	1	1	1	1	1	0	1	1
S40	0	1	1	1	0	1	0	0
S41	0	1	0	0	0	1	0	1
S42	0	1	0	0	0	0	0	0
S43	1	0	0	0	0	1	0	0
S44	0	1	0	0	0	1	0	0
S45	1	1	0	0	0	0	0	1
S46	0	0	1	1	1	1	1	0
S47	0	1	1	1	1	0	1	1
S48	1	0	1	1	1	0	1	0
S49	0	0	1	1	1	0	1	1
S50	1	0	1	0	1	1	0	0
S51	0	1	1	1	1	1	1	1
S52	1	1	1	1	1	0	0	1
S53	1	1	1	1	1	1	1	1
S54	0	0	1	0	1	0	1	0
S55	1	0	1	0	1	0	1	1

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Tetracladium breve*	*Tricladium angulatum*	*Articulospora tetracladia*	*Alatospora pulchella*	*Cylindrocladiella parva*	*Flagellospora leucorhynchos*	*Lunulospora curvula*	*Isthmolongispora lanceata*
S1	0	0	0	0	1	0	1	0
S2	0	0	1	0	0	0	0	0
S3	0	1	1	0	1	0	1	1
S4	0	0	1	0	0	0	0	0
S5	1	1	1	0	0	0	1	0
S6	0	0	1	0	0	1	0	0
S7	1	0	1	0	0	0	0	0
S8	0	0	1	0	0	0	0	0
S9	0	0	1	0	0	0	0	0
S10	1	1	0	1	0	1	1	1
S11	0	0	1	0	0	0	0	1
S12	0	1	0	1	0	0	0	0
S13	0	0	1	0	0	1	0	1
S14	0	0	0	0	0	0	1	0
S15	0	0	1	0	0	0	0	0
S16	0	0	1	1	0	0	0	1
S17	0	0	1	0	0	0	0	0
S18	0	1	1	0	1	0	0	0
S19	0	1	0	0	0	0	0	0
S20	0	0	0	0	1	0	0	0
S21	0	0	0	0	1	0	0	0
S22	0	0	0	0	0	0	0	0
S23	1	0	0	0	0	0	0	0
S24	0	0	1	0	1	0	0	0
S25	0	0	0	0	0	0	0	0
S26	1	0	0	0	0	0	0	0
S27	1	0	1	0	0	0	0	0
S28	0	1	0	1	0	1	0	0
S29	0	1	1	1	0	1	0	0
S30	1	1	0	1	0	1	1	1
S31	0	1	0	1	0	1	1	1
S32	1	1	1	1	0	1	0	0
S33	1	1	1	0	1	0	1	1
S34	1	1	0	1	0	1	0	0
S35	0	1	0	0	0	0	0	0
S36	1	1	0	1	0	1	1	0
S37	1	1	1	1	0	1	0	0
S38	1	1	1	1	0	0	0	0
S39	1	1	1	1	0	0	0	1
S40	0	0	0	0	1	1	0	0
S41	0	0	0	0	0	0	0	0
S42	1	0	0	0	0	0	0	0
S43	1	0	0	0	0	0	0	0
S44	0	0	0	0	0	0	0	0
S45	1	0	0	0	1	0	0	0
S46	1	1	1	0	0	1	1	0
S47	1	1	1	1	1	0	1	1
S48	0	0	0	0	1	0	1	1
S49	1	1	1	0	1	0	0	0
S50	1	1	0	0	1	0	0	0
S51	1	1	0	1	0	1	1	1
S52	0	1	0	0	0	0	0	0
S53	0	1	0	1	1	0	1	0
S54	0	0	0	0	0	0	1	1
S55	0	1	0	0	0	1	0	1

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Tetrachaetum elegans*	*Colispora cavincola*	*Neopyrenochaeta annellidica*	*Tumularia aquatica*	*Dactylella microaquatica*	*Dactylonectria macrodidyma*	*Vargamyces aquaticus*	*Filosporella annelidica*
S1	1	1	0	0	0	0	0	0
S2	0	0	0	0	1	0	0	1
S3	1	0	0	1	0	0	0	0
S4	0	0	0	0	0	0	0	1
S5	0	1	0	0	0	0	0	0
S6	0	0	0	0	0	0	0	0
S7	1	0	0	0	0	0	0	0
S8	1	0	0	0	0	0	0	0
S9	1	0	0	1	0	0	0	0
S10	0	0	0	1	0	1	0	0
S11	1	0	0	0	0	0	1	0
S12	0	0	1	1	0	0	0	0
S13	0	1	0	1	0	0	0	1
S14	1	1	1	0	0	0	0	0
S15	1	0	0	1	0	1	1	0
S16	1	0	0	1	0	1	0	0
S17	0	0	0	0	0	0	0	1
S18	0	0	0	0	0	0	0	1
S19	0	0	0	0	0	0	0	0
S20	0	0	0	0	0	0	0	0
S21	0	0	0	0	0	0	0	0
S22	0	0	0	0	0	0	0	0
S23	0	0	0	0	0	1	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	0	0	0	0	0	0
S26	0	0	0	0	0	0	0	0
S27	1	0	0	0	0	0	0	0
S28	0	0	0	1	1	0	0	1
S29	0	0	0	0	1	0	1	1
S30	0	0	0	1	1	0	0	0
S31	0	0	0	0	1	0	0	0
S32	0	0	1	0	0	0	1	1
S33	0	0	0	0	1	0	0	0
S34	0	0	0	0	1	0	0	0
S35	0	0	0	0	0	0	1	0
S36	0	0	1	1	1	1	0	0
S37	0	0	0	1	1	0	0	1
S38	0	1	0	0	1	0	0	0
S39	1	0	0	0	0	0	1	0
S40	0	0	0	0	0	1	0	0
S41	0	0	0	0	0	0	0	0
S42	0	0	0	0	0	1	0	0
S43	0	0	1	0	0	0	0	0
S44	0	0	0	0	0	0	0	0
S45	0	0	0	0	0	1	0	0
S46	0	1	1	0	1	0	1	0
S47	0	0	0	1	0	0	0	0
S48	1	1	0	0	0	0	0	0
S49	1	1	0	0	0	0	0	0
S50	0	1	1	0	0	0	1	0
S51	1	1	0	1	1	1	0	0
S52	0	0	1	0	0	0	1	0
S53	0	0	1	0	0	0	0	0
S54	0	1	1	0	0	1	1	0
S55	0	0	1	0	0	0	1	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Heliscella stellata*	*Lemonniera aquatica*	*Lemonniera cornuta*	*Taphrina sadebeckii*	*Aquanectria penicillioides*	*Tetracladium setigerum*	*Gyoerffyella entomobryoides*	*Mycoarthris corallina*
S1	0	0	1	0	1	0	0	0
S2	0	0	0	0	0	0	1	0
S3	0	0	0	0	0	0	0	1
S4	1	0	0	0	0	0	1	0
S5	0	0	0	0	0	0	0	0
S6	0	0	0	0	0	0	0	0
S7	0	0	1	1	0	0	1	0
S8	0	0	0	0	0	0	0	0
S9	0	0	0	0	0	0	0	0
S10	1	0	0	0	0	0	0	1
S11	0	1	0	0	0	0	1	0
S12	0	1	0	0	0	0	0	1
S13	1	0	0	0	0	0	1	0
S14	0	0	0	0	0	0	0	0
S15	0	0	0	0	0	0	0	0
S16	1	0	0	0	0	0	0	1
S17	0	0	0	0	0	0	0	0
S18	0	0	0	1	0	0	1	0
S19	0	0	0	0	0	0	0	0
S20	0	0	0	0	0	0	0	0
S21	0	0	0	0	0	0	0	0
S22	0	0	0	0	0	0	0	0
S23	0	0	0	0	0	0	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	0	0	1	0	0	0
S26	0	0	0	0	1	0	0	0
S27	0	1	1	1	0	0	1	0
S28	1	0	0	0	0	0	0	0
S29	1	0	0	0	0	0	0	0
S30	0	1	1	0	0	0	0	0
S31	0	0	0	1	0	0	0	0
S32	0	0	0	0	0	0	0	0
S33	0	0	1	1	0	1	0	0
S34	0	0	0	0	0	0	0	0
S35	0	0	0	0	0	0	0	0
S36	0	1	1	0	0	1	0	1
S37	0	0	0	0	0	0	0	0
S38	0	0	0	0	0	1	0	0
S39	0	0	0	0	0	0	0	0
S40	0	0	1	0	0	0	0	0
S41	0	0	0	0	0	0	0	0
S42	0	0	0	0	0	0	0	0
S43	0	0	0	0	0	1	0	0
S44	0	0	0	0	0	0	0	0
S45	0	0	0	1	0	1	0	0
S46	0	0	0	0	0	1	0	0
S47	1	0	0	0	0	0	0	1
S48	0	0	0	0	1	0	0	0
S49	0	0	0	0	0	0	0	0
S50	0	0	0	0	0	0	0	0
S51	0	1	1	0	0	0	0	0
S52	1	0	0	0	0	0	0	0
S53	0	0	0	1	0	0	0	0
S54	0	0	0	0	1	0	0	0
S55	0	1	1	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Neopyrenochaeta maesuayensis*	*Tricladium splendens*	*Arxiella terrestris*	*Boeremia galiicola*	*Dimorphospora foliicola*	*Plectosphaerella cucumerina*	*Alternaria alternata*	*Apiotrichum porosum*
S1	0	0	0	0	0	0	0	0
S2	0	0	0	0	0	0	0	0
S3	0	0	0	0	0	0	0	0
S4	0	0	0	0	0	0	0	0
S5	0	1	0	0	0	0	0	0
S6	0	0	0	0	0	0	1	0
S7	0	0	0	0	0	0	0	0
S8	0	0	0	0	0	0	0	0
S9	0	0	0	0	0	0	0	0
S10	0	0	1	0	0	1	0	1
S11	0	0	0	0	0	0	0	0
S12	0	0	1	0	0	0	0	0
S13	0	1	0	0	0	0	0	0
S14	1	0	0	0	0	0	0	0
S15	0	1	0	1	0	0	0	0
S16	0	1	0	0	1	1	0	0
S17	0	0	0	1	0	0	0	0
S18	0	0	0	0	0	0	0	0
S19	0	0	0	0	0	0	0	0
S20	0	0	0	0	1	0	0	1
S21	0	0	0	0	0	0	0	0
S22	0	0	0	0	0	0	0	0
S23	0	0	0	0	0	0	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	1	0	0	0	0	0
S26	0	0	1	0	0	1	0	0
S27	0	0	0	0	0	0	0	0
S28	0	0	0	0	0	0	0	0
S29	0	1	0	0	1	0	0	0
S30	0	0	1	0	0	0	0	0
S31	0	0	0	0	0	0	0	0
S32	0	0	0	1	0	0	0	1
S33	0	0	0	1	0	0	0	0
S34	0	0	0	0	0	0	0	0
S35	0	0	0	0	0	0	0	0
S36	0	0	0	0	0	0	0	0
S37	0	0	0	0	0	0	0	0
S38	0	1	0	0	1	0	0	0
S39	0	0	0	0	0	0	0	0
S40	0	0	0	0	0	0	1	0
S41	0	0	0	0	0	0	1	0
S42	0	0	0	0	0	0	0	0
S43	0	0	0	0	0	0	0	0
S44	0	0	0	0	0	0	1	0
S45	0	0	0	0	1	0	0	0
S46	0	0	0	0	0	0	0	0
S47	0	1	0	0	0	1	0	0
S48	1	0	0	0	0	0	0	0
S49	0	0	0	0	0	0	0	0
S50	0	0	0	0	0	0	0	0
S51	0	0	0	1	0	0	0	0
S52	0	0	0	0	0	0	0	0
S53	1	0	0	0	0	0	0	0
S54	1	0	0	0	0	0	0	0
S55	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Flagellospora fusarioides*	*Neonectria lugdunensis*	*Vishniacozyma heimaeyensis*	*Coprinellus micaceus*	*Paraphoma chrysanthemicola*	*Sydowia polyspora*	*Trisceloph orus cf. Acuminatus*	*Tumularia tuberculata*
S1	0	0	1	0	0	1	1	0
S2	0	0	0	0	0	0	0	0
S3	0	0	0	0	0	0	0	0
S4	0	0	0	0	0	0	0	0
S5	0	0	0	0	0	0	0	0
S6	1	0	0	0	0	0	0	1
S7	0	0	0	0	0	0	0	0
S8	0	0	0	0	0	0	0	0
S9	0	0	0	0	0	0	0	0
S10	0	1	0	0	0	0	0	0
S11	0	0	0	0	0	0	0	0
S12	0	0	0	0	0	0	0	0
S13	0	0	0	0	0	0	0	1
S14	0	0	0	0	0	0	1	0
S15	0	0	0	0	0	0	0	0
S16	0	0	0	0	0	0	0	0
S17	0	0	0	0	0	0	0	0
S18	0	0	0	0	0	0	0	0
S19	0	0	0	0	0	0	0	0
S20	0	1	0	0	0	0	0	0
S21	0	1	0	0	0	0	0	0
S22	0	0	0	0	0	0	0	0
S23	0	0	0	0	0	0	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	0	0	0	0	0	0
S26	0	0	0	0	0	0	0	0
S27	0	0	0	0	0	0	0	0
S28	0	1	0	0	0	0	0	0
S29	0	0	0	0	0	0	0	0
S30	0	0	0	0	0	0	0	0
S31	1	0	0	0	0	0	0	1
S32	0	0	1	1	0	0	0	0
S33	0	0	0	0	0	0	0	0
S34	0	0	0	0	0	0	0	0
S35	0	0	0	0	0	0	0	0
S36	1	0	0	0	0	0	0	0
S37	0	0	0	0	0	0	0	0
S38	0	0	0	0	0	0	0	0
S39	0	0	0	0	0	0	0	0
S40	0	1	0	0	1	0	0	0
S41	0	0	0	0	0	0	0	0
S42	0	0	0	0	0	0	0	0
S43	0	0	0	0	0	1	0	0
S44	0	0	0	0	0	0	0	0
S45	0	0	0	0	0	1	0	0
S46	0	0	0	0	0	0	0	1
S47	1	0	0	1	0	0	0	0
S48	0	0	1	0	0	0	1	0
S49	0	0	0	0	1	0	0	0
S50	0	0	0	0	0	0	0	0
S51	0	0	0	0	0	0	0	0
S52	0	0	0	1	0	0	0	0
S53	0	0	0	0	1	0	0	0
S54	0	0	1	1	0	0	0	0
S55	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Alternaria longipes*	*Alternaria tenuissima*	*Cylindrodendrum alicantinum*	*Cylindrodendrum hubeiense*	*Dactylonectria torresensis*	*Dendryphion nanum*	*Filobasidium globisporum*	*Fusarium acuminatum*
S1	0	0	0	0	0	0	0	0
S2	0	0	0	0	0	0	0	0
S3	0	0	0	0	0	0	0	0
S4	0	0	0	0	0	0	0	0
S5	0	0	0	0	0	0	0	0
S6	0	1	0	0	0	0	0	0
S7	0	0	0	0	0	0	0	0
S8	0	0	0	0	0	0	0	0
S9	0	0	0	0	0	0	0	0
S10	0	0	0	0	0	0	0	0
S11	0	0	0	0	0	0	1	0
S12	0	0	0	0	1	0	0	0
S13	0	0	0	0	0	0	0	0
S14	0	0	0	0	0	0	0	1
S15	0	0	0	0	0	0	0	0
S16	0	0	0	0	0	0	0	0
S17	0	0	0	0	0	0	0	0
S18	0	0	0	0	0	0	0	0
S19	0	0	0	0	0	0	0	0
S20	0	0	0	0	0	0	0	0
S21	0	0	0	0	0	0	0	0
S22	1	0	0	0	0	0	0	1
S23	0	0	0	0	0	0	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	0	0	0	0	0	0
S26	0	0	0	0	0	0	0	0
S27	0	0	0	0	0	0	0	0
S28	0	0	0	0	0	0	0	0
S29	0	0	0	1	0	0	0	0
S30	0	0	0	0	0	0	0	0
S31	0	0	0	0	0	0	0	0
S32	0	0	0	0	0	0	1	0
S33	0	0	0	0	0	0	0	0
S34	0	0	0	1	0	0	0	0
S35	1	0	0	0	0	0	0	0
S36	0	0	0	0	0	0	0	0
S37	0	0	0	0	0	0	0	0
S38	0	0	0	0	0	0	0	0
S39	0	0	0	0	0	0	0	0
S40	0	0	1	0	0	0	0	0
S41	0	0	0	0	1	0	0	0
S42	0	0	1	0	0	0	0	0
S43	0	0	0	0	0	0	0	0
S44	0	1	0	0	0	0	0	0
S45	0	0	0	0	0	0	0	1
S46	0	0	0	0	0	0	0	0
S47	0	0	0	0	0	0	0	0
S48	0	0	0	0	0	0	0	0
S49	0	0	0	0	0	0	0	0
S50	0	0	0	0	0	0	0	0
S51	0	0	0	0	0	0	0	0
S52	0	0	0	0	0	1	0	0
S53	0	1	0	0	0	1	1	0
S54	0	0	0	0	0	0	0	0
S55	0	0	0	0	0	1	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Geniculosporainflata*	*Gyoerffyella rotula*	*Ilyonectria robusta*	*Lemonniera pseudofloscula*	*Neopyrenochaeta acicola*	*Plectosphaerella plurivora*	*Sterkiella nova*	*Alternaria abundans*
S1	0	0	0	0	0	0	0	0
S2	0	0	0	1	0	0	0	0
S3	0	0	0	0	0	0	0	0
S4	0	1	0	0	0	0	0	0
S5	0	0	0	0	0	0	0	0
S6	0	0	0	0	0	0	0	0
S7	0	0	0	0	0	0	0	0
S8	0	1	0	0	0	0	0	0
S9	0	0	0	0	0	0	0	0
S10	0	0	0	0	0	1	0	0
S11	0	0	0	0	0	0	0	0
S12	0	0	0	0	0	0	0	0
S13	0	0	0	0	0	0	0	0
S14	0	0	0	0	0	0	0	0
S15	0	0	0	0	0	0	0	0
S16	0	0	0	0	0	0	0	0
S17	0	0	0	0	0	0	0	1
S18	0	0	0	0	0	0	0	0
S19	0	0	0	0	0	0	0	0
S20	0	0	0	0	0	0	0	0
S21	0	0	0	0	0	0	0	0
S22	0	0	0	0	0	0	0	0
S23	0	0	0	0	0	0	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	0	0	0	0	0	0
S26	0	0	0	0	0	0	0	0
S27	0	0	0	0	0	0	0	0
S28	0	0	0	0	0	0	0	0
S29	0	0	0	1	0	0	0	0
S30	0	0	0	0	0	0	0	0
S31	0	0	0	0	0	0	0	0
S32	1	0	0	0	0	0	0	0
S33	0	0	0	0	0	0	0	0
S34	0	0	0	0	0	0	0	0
S35	1	0	0	0	0	0	0	1
S36	0	0	1	0	0	0	0	0
S37	0	0	0	0	0	0	0	0
S38	1	0	0	0	0	0	0	0
S39	0	0	0	0	0	0	0	0
S40	0	0	0	0	0	0	0	0
S41	0	0	1	0	0	0	0	0
S42	0	0	0	0	0	0	0	0
S43	0	0	0	0	0	0	0	0
S44	0	0	0	0	0	0	0	0
S45	0	0	0	0	0	0	0	0
S46	0	0	0	0	0	0	0	0
S47	0	0	0	1	0	0	0	0
S48	0	0	0	0	0	0	0	0
S49	0	0	0	0	1	0	0	0
S50	0	0	0	0	0	0	0	0
S51	0	1	0	0	0	0	0	0
S52	0	0	0	0	0	0	1	0
S53	0	0	0	0	0	0	0	0
S54	0	0	0	0	0	0	0	0
S55	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Alternaria brassicae*	*Alternaria infectoria*	*Alternaria rosae*	*Alternaria triticina*	*Amniculicola lignicola*	*Boeremia exigua var. exigua*	*Cadophora luteo-olivacea*	*Curvularia coatesiae*
S1	0	0	0	0	0	0	0	0
S2	0	0	0	0	0	0	0	0
S3	0	0	0	0	0	0	0	0
S4	0	0	0	0	0	0	0	1
S5	0	0	0	0	0	0	0	0
S6	0	0	1	0	0	0	0	0
S7	0	0	0	0	0	0	0	0
S8	0	0	0	0	0	0	0	0
S9	0	0	0	0	0	1	0	0
S10	0	0	0	0	0	0	0	0
S11	0	0	0	0	0	0	0	0
S12	0	0	0	0	0	0	0	0
S13	0	0	0	0	0	0	0	0
S14	0	0	0	0	0	0	0	0
S15	0	0	0	0	0	0	0	0
S16	0	0	0	0	0	0	1	0
S17	0	0	0	0	0	0	0	0
S18	0	0	0	0	0	0	0	0
S19	0	0	0	0	0	0	0	0
S20	0	1	0	0	0	0	0	0
S21	0	0	0	0	0	0	0	0
S22	0	0	0	0	0	0	0	0
S23	0	0	0	0	0	0	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	0	0	0	0	0	0
S26	0	0	0	0	0	0	0	0
S27	0	0	0	0	0	0	0	0
S28	0	0	0	0	0	0	0	0
S29	0	0	0	0	0	0	0	0
S30	0	0	0	0	0	0	0	0
S31	0	0	0	0	0	0	0	0
S32	0	0	0	0	0	0	0	0
S33	0	0	0	0	0	0	0	0
S34	0	0	0	0	0	0	0	0
S35	0	0	0	0	0	0	0	1
S36	0	0	0	0	1	0	0	0
S37	0	0	0	0	0	0	0	0
S38	0	0	0	0	0	0	1	0
S39	0	0	0	0	0	0	0	0
S40	0	0	0	0	0	0	0	0
S41	0	0	0	0	0	0	0	0
S42	0	0	0	0	0	0	0	0
S43	0	1	0	0	0	0	0	0
S44	0	0	1	0	0	0	0	0
S45	0	0	0	0	0	0	0	0
S46	0	0	0	0	0	0	0	0
S47	0	0	0	0	0	0	0	0
S48	0	0	0	0	0	0	0	0
S49	0	0	0	0	0	0	0	0
S50	0	0	0	0	0	0	0	0
S51	0	0	0	0	0	0	0	0
S52	0	0	0	0	0	0	0	0
S53	0	0	0	0	0	1	0	0
S54	0	0	0	0	0	0	0	0
S55	1	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Fusarium oxysporum*	*Fusarium sporotrichioides*	*Helicodendron triglitziense*	*Hymenoscyphus cf. imberbis*	*Leptodontidium trabinellum*	*Neopyrenochaeta telephoni*	*Psychrophila olivacea*	*Pyrenochaetopsis leptospora*
S1	0	0	0	0	0	0	0	0
S2	0	1	0	0	0	0	0	0
S3	0	0	0	0	0	0	0	0
S4	0	0	0	0	0	0	0	0
S5	0	0	0	0	0	0	0	0
S6	0	0	0	0	0	0	0	0
S7	0	0	0	0	0	0	0	0
S8	0	1	0	0	0	0	0	0
S9	0	0	0	0	0	0	0	0
S10	0	0	0	0	1	0	0	0
S11	0	0	0	0	0	0	0	0
S12	0	0	0	1	0	0	0	0
S13	0	0	0	0	0	0	1	0
S14	0	0	0	0	0	0	0	1
S15	0	0	0	0	0	0	0	0
S16	0	0	0	1	0	0	0	0
S17	0	0	1	0	0	0	0	0
S18	0	0	0	0	0	0	0	0
S19	0	0	0	0	0	0	0	0
S20	0	0	0	0	0	0	0	0
S21	0	0	0	0	0	0	0	0
S22	0	0	0	0	0	0	0	0
S23	0	0	0	0	0	0	0	0
S24	0	0	0	0	0	0	0	0
S25	0	0	0	0	0	0	0	0
S26	0	0	0	0	0	0	0	0
S27	0	0	0	0	0	0	0	0
S28	0	0	0	0	0	0	0	0
S29	0	0	0	0	0	0	0	0
S30	0	0	0	0	0	0	1	0
S31	0	0	0	0	0	0	0	0
S32	0	0	0	0	0	0	0	0
S33	0	0	0	0	0	0	0	0
S34	0	0	0	0	0	0	0	0
S35	0	0	0	0	0	0	0	0
S36	0	0	0	0	0	0	0	0
S37	0	0	0	0	0	0	0	0
S38	0	0	0	0	0	0	0	0
S39	0	0	0	0	0	0	0	0
S40	0	0	0	0	0	0	0	0
S41	0	0	0	0	0	0	0	0
S42	0	0	0	0	0	0	0	0
S43	0	0	0	0	0	0	0	0
S44	0	0	0	0	0	0	0	0
S45	0	0	0	0	0	0	0	0
S46	0	0	0	0	0	0	0	0
S47	0	0	1	0	1	0	0	0
S48	0	0	0	0	0	0	0	0
S49	0	0	0	0	0	0	0	0
S50	0	0	0	0	0	0	0	0
S51	0	0	0	0	0	0	0	0
S52	0	0	0	0	0	0	0	0
S53	0	0	0	0	0	0	0	0
S54	0	0	0	0	0	0	0	0
S55	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Septoriella oudemansii*	*Sterkiella histriomuscorum*	*Taphrina alni*	*Tetracladium furcatum*
S1	0	0	0	0
S2	0	0	0	0
S3	0	0	0	0
S4	0	0	0	0
S5	0	0	0	0
S6	0	0	0	0
S7	0	0	0	0
S8	0	0	0	0
S9	0	0	0	0
S10	0	0	0	0
S11	0	0	0	0
S12	0	0	0	0
S13	0	0	0	0
S14	0	0	0	0
S15	0	0	0	0
S16	0	0	0	0
S17	0	0	0	0
S18	0	0	0	0
S19	0	0	0	0
S20	0	0	0	0
S21	0	0	0	0
S22	0	0	0	0
S23	0	0	0	0
S24	0	0	0	0
S25	0	0	0	0
S26	0	0	0	0
S27	0	0	0	0
S28	0	0	0	0
S29	0	0	0	0
S30	0	0	0	0
S31	0	0	0	0
S32	0	0	0	0
S33	1	0	0	0
S34	0	0	0	0
S35	0	0	0	0
S36	0	0	0	1
S37	0	0	0	0
S38	0	0	1	0
S39	0	0	0	0
S40	0	0	0	0
S41	0	0	0	0
S42	0	0	0	0
S43	0	0	0	1
S44	0	0	0	0
S45	0	0	0	0
S46	0	0	0	0
S47	0	0	0	0
S48	0	0	0	0
S49	0	0	0	0
S50	0	0	0	0
S51	0	0	0	0
S52	1	1	0	0
S53	0	0	0	0
S54	0	0	0	0
S55	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	Treat	Nutri	History	Site	*Tetracladium marchalianum*	*Clavariopsis aquatica*	*Alatospora acuminata*	*Amniculicola guttulata*	*Margaritispora aquatica*	*Flagellospora curvula*
S56	30	HIGH	W	B	1	1	1	1	0	1
S57	30	LOW	W	B	1	1	1	1	1	1
S58	30	HIGH	W	H	1	1	0	1	1	0
S59	30	VLOW	W	B	1	1	1	1	0	1
S60	30	LOW	W	E	1	1	0	1	0	1
S61	30	HIGH	W	E	1	1	1	1	1	1
S62	30	LOW	W	H	1	1	1	1	1	1
S63	30	VLOW	W	H	1	1	1	1	1	1
S64	300	LOW	W	E	1	0	0	0	1	1
S65	300	VLOW	W	B	1	0	0	0	0	0
S66	300	LOW	W	B	1	0	0	0	0	0
S67	300	HIGH	W	B	1	0	0	0	1	1
S68	300	HIGH	W	E	1	0	0	0	1	0
S69	300	VLOW	W	H	1	0	0	0	1	0
S70	300	LOW	W	H	1	1	0	0	0	1
S71	300	VLOW	W	E	1	0	0	0	1	1
S72	300	HIGH	W	H	1	1	0	1	1	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Aureobasidium pullulans*	*Epicoccum nigrum*	*Amniculicola longissima*	*Pithomyces chartarum*	*Clavatospora longibrachiata*	*Pseudopithomyces palmicola*	*Stenocladiella neglecta*	*Lemonniera terrestris*
S56	0	0	1	1	1	1	1	0
S57	1	1	1	1	1	1	1	0
S58	0	1	0	0	1	0	1	0
S59	1	0	1	1	1	1	0	0
S60	1	1	0	1	1	0	0	0
S61	1	1	1	1	1	1	1	0
S62	1	0	0	1	0	1	1	1
S63	1	0	0	0	0	0	1	0
S64	1	1	0	1	0	1	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	1	1	0	0	0	0	0
S68	0	0	0	0	0	0	0	0
S69	1	0	0	0	1	0	0	0
S70	0	0	0	0	0	0	0	1
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Tetracladium breve*	*Tricladium angulatum*	*Articulospora tetracladia*	*Alatospora pulchella*	*Cylindrocladiella parva*	*Flagellospora leucorhynchos*	*Lunulospora curvula*	*Isthmolongispora lanceata*
S56	0	1	1	1	0	1	0	0
S57	1	1	0	1	0	0	0	0
S58	0	0	0	1	1	0	1	0
S59	1	1	0	1	0	1	0	1
S60	1	0	0	1	0	0	0	1
S61	1	0	0	0	0	0	0	0
S62	1	0	1	1	1	1	1	1
S63	0	0	0	0	1	0	1	0
S64	1	0	0	0	0	0	0	0
S65	0	0	0	0	1	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	0	0	0	0	0
S68	1	0	0	0	1	0	0	0
S69	0	0	0	0	0	0	0	0
S70	1	0	0	0	1	0	0	0
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Tetrachaetum elegans*	*Colispora cavincola*	*Neopyrenochaeta annellidica*	*Tumularia aquatica*	*Dactylella microaquatica*	*Dactylonectria macrodidyma*	*Vargamyces aquaticus*	*Filosporella annelidica*
S56	0	1	1	1	1	0	0	0
S57	0	0	0	1	1	0	0	0
S58	0	1	1	0	0	0	0	0
S59	1	1	1	1	0	1	1	1
S60	0	0	1	0	0	0	1	0
S61	0	1	1	0	0	0	0	0
S62	0	0	0	0	0	0	0	1
S63	1	1	0	0	1	0	0	0
S64	0	0	0	0	0	0	0	0
S65	1	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	0	0	1	0	0
S68	0	0	0	0	0	0	0	0
S69	0	0	0	0	0	1	0	0
S70	0	0	0	0	0	0	0	0
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Heliscella stellata*	*Lemonniera aquatica*	*Lemonniera cornuta*	*Taphrina sadebeckii*	*Aquanectria penicillioides*	*Tetracladium setigerum*	*Gyoerffyella entomobryoides*	*Mycoarthris corallina*
S56	0	1	0	0	0	0	0	0
S57	0	0	0	0	0	1	0	0
S58	0	0	0	0	1	0	0	0
S59	1	1	0	0	0	0	0	0
S60	0	1	0	0	0	0	0	0
S61	0	1	0	1	0	0	0	0
S62	1	0	0	0	0	1	0	0
S63	1	0	0	0	0	0	0	0
S64	0	0	0	0	0	0	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	0	0	0	0	0
S68	0	0	0	0	0	0	0	1
S69	0	0	0	1	1	0	0	0
S70	0	0	0	0	0	0	0	0
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	1	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Neopyrenochaeta maesuayensis*	*Tricladium splendens*	*Arxiella terrestris*	*Boeremia galiicola*	*Dimorphospora foliicola*	*Plectosphaerella cucumerina*	*Alternaria alternata*	*Apiotrichum porosum*
S56	1	0	0	0	0	0	0	0
S57	0	0	0	0	0	0	0	0
S58	1	0	0	0	0	1	0	0
S59	0	0	0	0	0	0	0	1
S60	0	0	0	0	0	0	1	0
S61	1	0	0	0	0	0	0	0
S62	0	0	0	1	0	1	0	0
S63	0	0	0	0	1	0	0	0
S64	0	0	0	0	0	0	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	1	0	0	0	0	0
S68	0	0	0	0	0	0	0	0
S69	0	0	0	0	0	0	0	0
S70	0	0	0	0	0	0	0	1
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Flagellospora fusarioides*	*Neonectria lugdunensis*	*Vishniacozyma heimaeyensis*	*Coprinellus micaceus*	*Paraphoma chrysanthemicola*	*Sydowia polyspora*	*Trisceloph orus cf. Acuminatus*	*Tumularia tuberculata*
S56	0	0	0	0	0	0	0	0
S57	0	0	0	0	0	0	0	0
S58	0	0	0	0	0	0	0	0
S59	0	0	0	0	1	0	0	0
S60	0	0	0	0	0	0	0	0
S61	0	0	0	0	0	1	0	0
S62	1	0	0	0	0	0	0	0
S63	0	0	1	0	0	0	1	0
S64	0	0	0	0	0	0	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	0	0	0	0	0
S68	0	0	0	0	0	0	0	0
S69	0	0	0	0	0	0	0	0
S70	0	0	0	0	0	0	0	0
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Alternaria longipes*	*Alternaria tenuissima*	*Cylindrodendrum alicantinum*	*Cylindrodendrum hubeiense*	*Dactylonectria torresensis*	*Dendryphion nanum*	*Filobasidium globisporum*	*Fusarium acuminatum*
S56	0	0	1	0	1	0	0	0
S57	1	0	0	0	0	0	0	0
S58	0	0	0	0	0	0	0	0
S59	0	0	0	0	0	0	0	0
S60	0	0	0	0	0	0	0	0
S61	0	0	0	0	0	0	0	0
S62	0	0	0	0	0	0	0	0
S63	0	0	0	0	0	0	0	0
S64	0	0	0	0	0	0	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	1	0	0	0	0
S68	0	0	0	0	0	0	0	0
S69	0	0	0	0	0	0	0	0
S70	0	0	0	0	0	0	0	0
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Geniculospora inflata*	*Gyoerffyella rotula*	*Ilyonectria robusta*	*Lemonniera pseudofloscula*	*Neopyrenochaeta acicola*	*Plectosphaerella plurivora*	*Sterkiella nova*	*Alternaria abundans*
S56	0	0	0	0	0	0	0	0
S57	0	0	0	0	0	0	0	0
S58	0	0	0	0	1	0	0	0
S59	0	0	0	0	0	0	0	0
S60	0	0	0	0	0	0	0	0
S61	0	0	0	0	0	1	1	0
S62	0	0	0	0	0	1	0	0
S63	0	0	0	0	0	0	0	0
S64	0	0	0	0	0	0	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	0	0	0	0	0
S68	0	0	0	0	0	0	1	0
S69	0	0	0	0	0	0	0	0
S70	0	0	1	0	0	0	0	0
S71	0	0	0	0	0	0	0	0
S72	0	0	0	0	1	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Alternaria brassicae*	*Alternaria infectoria*	*Alternaria rosae*	*Alternaria triticina*	*Amniculicola lignicola*	*Boeremia exigua var. exigua*	*Cadophora luteo-olivacea*	*Curvularia coatesiae*
S56	0	0	0	0	1	0	0	0
S57	0	0	0	1	0	0	0	0
S58	0	0	0	0	0	0	0	0
S59	0	0	0	0	0	0	0	0
S60	0	0	0	0	0	0	0	0
S61	0	0	0	0	0	0	0	0
S62	0	0	0	0	0	0	0	0
S63	0	0	0	0	0	0	0	0
S64	0	0	0	0	0	0	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	0	0	0	0	0
S68	0	0	0	0	0	0	0	0
S69	0	0	0	0	0	0	0	0
S70	1	0	0	0	0	0	0	0
S71	0	0	0	1	0	0	0	0
S72	0	0	0	0	0	0	0	0

Table S7 – Presence-absence table from the 93 species used to characterize fungal community and differences among treatments- continuation.

SAMPLE	*Fusarium oxysporum*	*Fusarium sporotrichioides*	*Helicodendron triglitziense*	*Hymenoscyphus cf. imberbis*	*Leptodontidium trabinellum*	*Neopyrenochaeta telephoni*	*Psychrophila olivacea*	*Pyrenochaetopsis leptospora*
S56	0	0	0	0	0	1	0	0
S57	0	0	0	0	0	0	0	0
S58	0	0	0	0	0	0	0	0
S59	0	0	0	0	0	0	0	0
S60	0	0	0	0	0	1	0	0
S61	0	0	0	0	0	0	0	0
S62	0	0	0	0	0	0	0	0
S63	0	0	0	0	0	0	0	1
S64	0	0	0	0	0	0	0	0
S65	0	0	0	0	0	0	0	0
S66	0	0	0	0	0	0	0	0
S67	0	0	0	0	0	0	0	0
S68	1	0	0	0	0	0	0	0
S69	0	0	0	0	0	0	0	0
S70	0	0	0	0	0	0	0	0
S71	0	0	0	0	0	0	0	0
S72	1	0	0	0	0	0	0	0

Table S8 – Dose- response model parameters used for Fig. 1. P: pristine; W:wastewater V: vineyard run-off.

Nutrient level	Community History	Model	Lower limit	Parameters			
Very low	P	Log-logistic (ED_{50} as parameter)	0	b:0.280;	d:0.021;	e:1.709 x10^{11}	
	W	Log-normal	0	b:0.013;	d:0.043;	e:0.002	
	V	Weibull (type 1)	0	b:0.261;	d:0.023;	e: 85991	
Low	P	Cedergreen-Ritz-Streibig (alpha=0.25)	0	b:0.12;	d:0.025;	e:0.014	f:0.105
	W	Log-logistic (ED50 as parameter)	0	b:1.262;	d:0.025;	e:2.400 x10^{5}	
	V	Log-logistic (ED50 as parameter)	0	c:0.078;	d:0.030;	e:1.737 x10^{14}	
Mod	P	Log-logistic (ED50 as parameter)	0	b:2.292;	d:0.027;	e:27062	
	W	Log-logistic (ED50 as parameter)	0	b:5.047;	d:0.029;	e:271.360	
	V	Log-logistic (ED50 as parameter)	0	b:0.217;	d:0.031;	e:1.90x 10^{21}	
High	P	Log-normal	0	c:-0.877;	d:0.032;	e:1366.600	
	W	Cedergreen-Ritz-Streibig (alpha=1)	0	b: 1.694;	d:0.030;	e:1667.9	f:0.004
	V	Log-logistic (log(ED50) as parameter)	0	b:0.123;	d:0.032;	e:31.050	

Table S9 - Surface plot model parameters used for figure S1. P: pristine; W: wastewater V: vineyard run-off

Community History	Nutriel level	Model	Parameters			
P	Vlow	Shifted Michaelis-Menten (3 parms)	c:0.021;	d:0.039;	e:2.344x 10^{10}	
	Low	Shifted Michaelis-Menten (3 parms)	c:0.024;	d:0.025;	e:0.011	
	Mod	Shifted Michaelis-Menten (3 parms)	c:0.025;	d:0.030;	e:45.997	
	high	Log-normal with lower limit at 0 (3 parms)	c:-0.877;	d:0.032;	e:1366.6	
W	Vlow	Shifted Michaelis-Menten (3 parms)	c: 0.024	d:0.023;	e:3.463	
	Low	Shifted Michaelis-Menten (3 parms)	c:0.025;	d:0.064;	e:24845	
	Mod	Shifted Michaelis-Menten (3 parms)	c:0.028;	d:0.055;	e:1660.2	
	high	Log-logistic (log(ED50) as parameter) (4 parms)	b:4.6806 x10^{-05};	c:0.038;	d: 0.031	e:12.519
V	Vlow	Shifted Michaelis-Menten (3 parms)	c:0.023;	d:0.019;	e:1.317	
	Low	Weibull (type 1) with lower limit at 0 (3 parms)	c:0.078;	d:0.029;	e:1.537x 10^{14}	
	Mod	Log-logistic (ED50 as parameter) with upper limit at 1 (3 parms)	c:-0.827;	d:0.031;	e:1.235x 10^{21}	
	high	Shifted Michaelis-Menten (3 parms)	c:0.031;	d:0.030;	e:17.657	

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Microbial breakdown rate (k)				
			mean	sd	ci	variation to control %	variation to Pristine %
P	0	V_Low	0.02045	0.00216	0.00189		
		Low	0.02456	0.00513	0.00449	16.7	
		Mod	0.0242	0.00289	0.00253	15.5	
		High	0.02996	0.00289	0.00253	31.8	
	3	V_Low	0.0215	0.00201	0.00176	4.9	
		Low	0.02564	0.00488	0.00428	20.2	
		Mod	0.02633	0.0026	0.00228	22.3	
		High	0.03263	0.00375	0.00329	37.3	
	30	V_Low	0.02154	0.00484	0.00424	5	
		Low	0.02414	0.00422	0.0037	15.3	
		Mod	0.02674	0.005	0.00439	23.5	
		High	0.03201	0.00362	0.00318	36.1	
	300	V_Low	0.02126	0.00412	0.00361	3.8	
		Low	0.02438	0.00505	0.00443	16.1	
		Mod	0.02947	0.00655	0.00574	30.6	
		High	0.02863	0.00682	0.00598	28.6	
			mean	sd	ci		
WWTP	0	V_Low	0.02358	0.00427	0.00374	-5.7	13.3
		Low	0.02491	0.00354	0.0031	0	1.4
		Mod	0.02801	0.00306	0.00269	11.1	13.6
		High	0.03026	0.00311	0.00272	17.7	1
	3	V_Low	0.0238	0.00199	0.00174	-4.7	9.6
		Low	0.02393	0.00089	0.00078	-4.1	-7.1
		Mod	0.02766	0.00224	0.00196	9.9	4.8
		High	0.03548	0.00376	0.0033	29.8	8
	30	V_Low	0.0233	0.00478	0.00419	-6.9	7.6
		Low	0.02545	0.00174	0.00152	2.1	5.1
		Mod	0.02748	0.00294	0.00258	9.4	2.7
		High	0.03318	0.00096	0.00084	24.9	3.5
	300	V_Low	0.02349	0.00409	0.00358	-6	9.5
		Low	0.02513	0.00412	0.00361	0.9	3
		Mod	0.0318	0.00431	0.00378	21.7	7.3
		High	0.03356	0.00502	0.0044	25.8	14.7
			mean	sd	ci		
VYRO	0	V_Low	0.01548	0.01095	0.0096	-26.7	-32.1
		Low	0.01961	0.01387	0.01216	0	-25.2
		Mod	0.02021	0.01429	0.01253	3	-19.7
		High	0.02096	0.01529	0.0134	6.4	-43
	3	V_Low	0.01374	0.00972	0.00852	-42.8	-56.5
		Low	0.01728	0.01253	0.01098	-13.5	-48.4
		Mod	0.01937	0.01384	0.01213	-1.2	-35.9
		High	0.02071	0.01472	0.0129	5.3	-57.5
	30	V_Low	0.01262	0.00934	0.00818	-55.5	-70.7
		Low	0.01916	0.01421	0.01246	-2.4	-26
		Mod	0.02193	0.01558	0.01366	10.6	-21.9
		High	0.02047	0.01501	0.01315	4.2	-56.3
	300	V_Low	0.0132	0.00981	0.0086	-48.6	-61.1
		Low	0.01684	0.01197	0.01049	-16.5	-44.8
		Mod	0.02165	0.01533	0.01343	9.4	-36.1
		High	0.02001	0.01469	0.01288	2	-43.1

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine - continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Recalcitrance ratio		
				variation to control %	variation to Pristine %
P	0	V_Low	0.060441		
		Low	0.046166	-30.9	
		Mod	0.058273	-3.7	
		High	0.056801	-6.4	
	3	V_Low	0.062084	2.6	
		Low	0.052594	-14.9	
		Mod	0.060332	-0.2	
		High	0.063216	4.4	
	30	V_Low	0.061773	2.2	
		Low	0.088258	31.5	
		Mod	0.057886	-4.4	
		High	0.064082	5.7	
	300	V_Low	0.071143	15	
		Low	0.058395	-3.5	
		Mod	0.06091	0.8	
		High	0.053681	-12.6	
WWTP	0	V_Low	0.049487	1.2	-22.1
		Low	0.048897	0	5.6
		Mod	0.061282	20.2	4.9
		High	0.058736	16.8	3.3
	3	V_Low	0.066228	26.2	6.3
		Low	0.0536	8.8	1.9
		Mod	0.045303	-7.9	-33.2
		High	0.044467	-10	-42.2
	30	V_Low	0.061617	20.6	-0.3
		Low	0.076654	36.2	-15.1
		Mod	0.073556	33.5	21.3
		High	0.062377	21.6	-2.7
	300	V_Low	0.050247	2.7	-41.6
		Low	0.05359	8.8	-9
		Mod	0.048612	-0.6	-25.3
		High	0.055892	12.5	4
VYRO	0	V_Low	0.05633	-5.4	-7.3
		Low	0.059394	0	22.3
		Mod	0.070223	15.4	17
		High	0.058663	-1.2	3.2
	3	V_Low	0.06529	9	4.9
		Low	0.053276	-11.5	1.3
		Mod	0.06195	4.1	2.6
		High	0.04891	-21.4	-29.2
	30	V_Low	0.072865	18.5	15.2
		Low	0.061291	3.1	-44
		Mod	0.065123	8.8	11.1
		High	0.05433	-9.3	-18
	300	V_Low	0.068104	12.8	-4.5
		Low	0.061002	2.6	4.3
		Mod	0.061425	3.3	0.8
		High	0.064987	8.6	17.4

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine - continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Number of bacterial DNA copies					
			mean	sd	ci		variation to control %	variation to Pristine %
P	0	V_Low	5.05E+08	2.32E+08	2.04E+08			
		Low	3.65E+09	4.9E+09	4.3E+09		86.2	
		Mod	5.67E+08	2.06E+08	1.8E+08		11	
		High	7.83E+08	1.37E+08	1.2E+08		35.6	
	3	V_Low	1.12E+09	2.97E+08	2.6E+08		54.7	
		Low	1.09E+09	1.28E+08	1.12E+08		53.6	
		Mod	1.52E+09	1.25E+09	1.09E+09		66.8	
		High	4.52E+08	33739997	29574411		-11.8	
	30	V_Low	1.69E+09	9.27E+08	8.13E+08		70.2	
		Low	4.38E+08	1.86E+08	1.63E+08		-15.3	
		Mod	4.76E+08	15759908	13814168		-6.1	
		High	4.44E+08	46022198	40340235		-13.6	
	300	V_Low	1.21E+09	1.24E+09	1.08E+09		58.3	
		Low	4.93E+08	3.64E+08	3.19E+08		-2.4	
		Mod	5.41E+08	3.01E+08	2.64E+08		6.8	
		High	2.74E+08	1.33E+08	1.17E+08		-84	
			mean	sd	ci			
WWTP	0	V_Low	2.07E+09	2.48E+09	2.17E+09		71	75.6
		Low	6.00E+08	1.12E+08	97901157		0	-507.3
		Mod	1.35E+09	9.64E+08	8.45E+08		55.6	58
		High	2.87E+09	1.74E+09	1.52E+09		79.1	72.7
	3	V_Low	4.10E+08	1.58E+08	1.39E+08		-46.6	-172.3
		Low	3.80E+08	1.13E+08	99122257		-58.1	-186.6
		Mod	8.65E+08	24637242	21595495		30.6	-75.6
		High	2.20E+09	1.01E+09	8.86E+08		72.7	79.5
	30	V_Low	9.05E+08	7.33E+08	6.42E+08		33.6	-87
		Low	4.47E+08	34953460	30638059		-34.4	2
		Mod	5.68E+08	88031077	77162641		-5.8	16.2
		High	7.32E+08	2.58E+08	2.26E+08		18	39.3
	300	V_Low	2.87E+08	22207129	19465407		-109.4	-321.6
		Low	1.21E+09	1.39E+09	1.21E+09		50.3	59.2
		Mod	4.80E+08	1.57E+08	1.38E+08		-25	-12.7
		High	6.80E+08	5.96E+08	5.22E+08		11.6	59.6
			mean	sd	ci			
VYRO	0	V_Low	4.79E+08	4.6E+08	4.03E+08		65.4	-5.5
		Low	1.66E+08	1.17E+08	1.03E+08		0	-2102.3
		Mod	3.06E+08	2.32E+08	2.03E+08		45.9	-85.3
		High	2.95E+08	2.11E+08	1.85E+08		43.9	-165.4
	3	V_Low	3.43E+08	3.84E+08	3.36E+08		51.7	-225.6
		Low	1.97E+08	2.1E+08	1.84E+08		15.8	-453.2
		Mod	9.21E+08	1.19E+09	1.04E+09		82	-65.1
		High	2.13E+08	2.19E+08	1.92E+08		22.3	-111.8
	30	V_Low	1.85E+08	1.53E+08	1.34E+08		10.6	-812.7
		Low	2.71E+08	2.19E+08	1.92E+08		38.8	-61.7
		Mod	4.10E+08	3.34E+08	2.93E+08		59.6	-16.1
		High	2.69E+08	2.82E+08	2.47E+08		38.5	-65.1
	300	V_Low	2.43E+08	2.46E+08	2.16E+08		32	-396.9
		Low	3.03E+08	2.71E+08	2.38E+08		45.4	-62.4
		Mod	1.59E+08	1.27E+08	1.11E+08		-4.3	-241.1
		High	92135997	68835213	60336724		-79.7	-197.8

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine - continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Number of Fungal DNA copies					
			mean	sd	ci		variation to control %	variation to Pristine %
P	0	V_Low	3.38E+09	1.17E+09	1.03E+09			
		Low	1.51E+10	1.92E+10	1.68E+10		93.9	
		Mod	4.07E+09	1.45E+09	1.27E+09		19	
		High	5.21E+09	5.47E+08	4.79E+08		-114.5	
	3	V_Low	6.47E+09	1.08E+09	9.45E+08		-8.8	
		Low	6.97E+09	86670565	75970100		-1253	
		Mod	8.29E+09	5.84E+09	5.12E+09		79.9	
		High	3.24E+09	1.63E+08	1.43E+08		-619.8	
	30	V_Low	9.08E+09	4.31E+09	3.78E+09		72.8	
		Low	3.15E+09	1.15E+09	1.01E+09		-1.6	
		Mod	3.45E+09	1.11E+08	97476945		-954.4	
		High	3.24E+09	3.65E+08	3.2E+08		-220.9	
	300	V_Low	5.97E+09	4.92E+09	4.31E+09		76.2	
		Low	3.12E+09	1.77E+09	1.55E+09		33.6	
		Mod	3.62E+09	1.54E+09	1.35E+09		24	
		High	2.13E+09	8.94E+08	7.84E+08		-31.1	
			mean	sd	ci			
WWTP	0	V_Low	9.62E+09	1.02E+10	8.91E+09		94.3	64.9
		Low	4.03E+09	5.82E+08	5.1E+08		0	-275.6
		Mod	7.19E+09	3.47E+09	3.04E+09		83.2	43.5
		High	1.30E+10	6.29E+09	5.52E+09		90.8	59.8
	3	V_Low	2.82E+09	7.9E+08	6.93E+08		26.3	-129.2
		Low	2.85E+09	7.3E+08	6.4E+08		20.3	-144.6
		Mod	5.62E+09	2.31E+08	2.02E+08		-152.1	-47.3
		High	1.23E+10	5.18E+09	4.54E+09		88.8	73.6
	30	V_Low	5.31E+09	3.55E+09	3.11E+09		83.6	-70.9
		Low	3.23E+09	3.96E+08	3.47E+08		-47	2.7
		Mod	3.98E+09	6.84E+08	5.99E+08		14.8	13.5
		High	4.93E+09	1.49E+09	1.3E+09		60.8	34.3
	300	V_Low	2.19E+09	2.33E+08	2.04E+08		-149.9	-173.1
		Low	5.84E+09	5.74E+09	5.03E+09		89.9	46.6
		Mod	3.22E+09	8.17E+08	7.16E+08		28.7	-12.5
		High	4.18E+09	3.07E+09	2.69E+09		81	49
			mean	sd	ci			
VYRO	0	V_Low	3.06E+09	2.77E+09	2.43E+09		65.1	-10.2
		Low	1.36E+09	9.68E+08	8.49E+08		0	-1011.2
		Mod	2.18E+09	1.58E+09	1.39E+09		38.7	-86.5
		High	2.21E+09	1.57E+09	1.38E+09		38.4	-136
	3	V_Low	2.10E+09	2.27E+09	1.99E+09		57.3	-208.2
		Low	1.48E+09	1.52E+09	1.33E+09		36.2	-371.4
		Mod	4.81E+09	5.8E+09	5.08E+09		83.3	-72.1
		High	1.61E+09	1.53E+09	1.35E+09		36.9	-101.8
	30	V_Low	1.46E+09	1.13E+09	9.93E+08		14.5	-521.7
		Low	1.96E+09	1.51E+09	1.32E+09		35.7	-60.7
		Mod	2.74E+09	2.09E+09	1.83E+09		53.7	-25.6
		High	1.80E+09	1.68E+09	1.47E+09		42.3	-80
	300	V_Low	1.75E+09	1.68E+09	1.47E+09		42.3	-241.2
		Low	2.00E+09	1.6E+09	1.41E+09		39.7	-56.2
		Mod	1.23E+09	9.27E+08	8.13E+08		-4.5	-193.5
		High	8.15E+08	5.99E+08	5.25E+08		-61.6	-161.5

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_b a-BGL					
			mean	sd	ci		variation to control %	variation to Pristine %
P	0	V_Low	8674.342	4686.70	4108.07			
		Low	13078.99	14112.78	12370.40		33.68	
		Mod	13141.43	8979.06	7870.49		33.99	
		High	14596.44	13625.37	11943.16		40.57	
	3	V_Low	8702.5	4935.65	4326.29		0.32	
		Low	11025.25	6334.34	5552.29		21.32	
		Mod	11625.11	9427.38	8263.46		25.38	
		High	11276.78	5535.77	4852.32		23.08	
	30	V_Low	7754.464	4622.26	4051.59		-11.86	
		Low	7903.299	4247.15	3722.80		-9.76	
		Mod	12192.01	6566.13	5755.47		28.85	
		High	11866.28	5181.72	4541.97		26.90	
	300	V_Low	9601.492	5619.26	4925.50		9.66	
		Low	8468.139	4546.71	3985.37		-2.44	
		Mod	8650.409	4557.50	3994.82		-0.28	
		High	12166.16	10039.48	8800.00		28.70	
			mean	sd	ci			
WWTP	0	V_Low	11716.51	6191.37	5426.98		-27.89	25.96
		Low	14984.3	12273.70	10758.37		0.00	12.72
		Mod	12028.2	8109.90	7108.64		-24.58	-9.26
		High	9595.676	5652.41	4954.56		-56.16	-52.11
	3	V_Low	11769.29	13143.59	11520.87		-27.32	26.06
		Low	7493.131	3110.65	2726.61		-99.97	-47.14
		Mod	9652.044	7833.73	6866.57		-55.24	-20.44
		High	11892.24	7802.67	6839.34		-26.00	5.18
	30	V_Low	12815.01	17314.62	15176.93		-16.93	39.49
		Low	7102.05	2738.24	2400.17		-110.99	-11.28
		Mod	9185.427	5396.88	4730.57		-63.13	-32.73
		High	9622.97	3348.84	2935.39		-55.71	-23.31
	300	V_Low	9253.99	5738.42	5029.95		-61.92	-3.76
		Low	9451.03	5581.57	4892.46		-58.55	10.40
		Mod	10014.75	3371.59	2955.33		-49.62	13.62
		High	9853.923	3385.04	2967.12		-52.06	-23.47
			mean	sd	ci			
VYRO	0	V_Low	10917.55	12327.50	10805.53		36.19	20.55
		Low	6966.924	5791.01	5076.04		0.00	-87.73
		Mod	11743.67	8009.51	7020.64		40.68	-11.90
		High	11148.64	6805.39	5965.18		37.51	-30.93
	3	V_Low	8465.668	4703.58	4122.87		17.70	-2.80
		Low	10998.42	8713.07	7637.34		36.66	-0.24
		Mod	10229.75	7835.11	6867.78		31.90	-13.64
		High	13098.71	7180.06	6293.60		46.81	13.91
	30	V_Low	6053.618	3137.03	2749.73		-15.09	-28.10
		Low	5114.612	2349.51	2059.44		-36.22	-54.52
		Mod	8961.382	6052.38	5305.14		22.26	-36.05
		High	9432.669	5519.51	4838.06		26.14	-25.80
	300	V_Low	9262.514	6233.45	5463.86		24.78	-3.66
		Low	11472.96	8618.50	7554.45		39.28	26.19
		Mod	10336.11	4586.95	4020.64		32.60	16.31
		High	11163.74	7656.35	6711.08		37.59	-8.98

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_c b-BGL					
			mean	sd	ci		variation to control %	variation to Pristine %
P	0	V_Low	75835.91	54173.98	47485.59			
		Low	82195.62	48114.35	42174.08		7.74	
		Mod	84746.32	61269.73	53705.29		10.51	
		High	81127.12	55207.52	48391.53		6.52	
	3	V_Low	94969.32	51615.38	45242.87		20.15	
		Low	73988.47	37876.96	33200.62		-2.50	
		Mod	70874.45	39056.51	34234.54		-7.00	
		High	72583.91	34958.16	30642.18		-4.48	
	30	V_Low	56885.14	39020.89	34203.32		-33.31	
		Low	66081.45	42596.37	37337.36		-14.76	
		Mod	94953.76	109417.98	95909.09		20.13	
		High	96701.40	82288.97	72129.46		21.58	
	300	V_Low	57630.37	35670.33	31266.43		-31.59	
		Low	57189.78	34227.87	30002.05		-32.60	
		Mod	50949.88	26336.11	23084.61		-48.84	
		High	58370.95	31546.24	27651.50		-29.92	
			mean	sd	ci			
WWTP	0	V_Low	77476.74	50119.62	43931.78		-25.55	2.12
		Low	97271.17	56240.59	49297.05		0.00	15.50
		Mod	79361.54	42825.67	37538.35		-22.57	-6.79
		High	83009.63	37877.84	33201.39		-17.18	2.27
	3	V_Low	82699.04	37162.76	32574.59		-17.62	-14.84
		Low	97189.85	52512.94	46029.62		-0.08	23.87
		Mod	85189.28	34368.96	30125.72		-14.18	16.80
		High	80735.77	24861.55	21792.11		-20.48	10.10
	30	V_Low	69153.87	73083.66	64060.65		-40.66	17.74
		Low	49483.73	33262.88	29156.20		-96.57	-33.54
		Mod	72967.27	14942.42	13097.61		-33.31	-30.13
		High	73015.94	24542.36	21512.33		-33.22	-32.44
	300	V_Low	65613.83	24555.63	21523.96		-48.25	12.17
		Low	57035.67	34927.39	30615.21		-70.54	-0.27
		Mod	70384.83	26203.25	22968.16		-38.20	27.61
		High	60414.47	13408.90	11753.42		-61.01	3.38
			mean	sd	ci			
VYRO	0	V_Low	65856.78	60431.56	52970.60		-3.00	-15.15
		Low	67834.71	46356.80	40633.52		0.00	-21.17
		Mod	77805.86	50002.01	43828.69		12.82	-8.92
		High	64674.56	30900.94	27085.87		-4.89	-25.44
	3	V_Low	75863.50	52290.28	45834.45		10.58	-25.18
		Low	84956.72	43328.96	37979.51		20.15	12.91
		Mod	84745.53	56510.40	49533.55		19.95	16.37
		High	66532.03	29549.94	25901.66		-1.96	-9.10
	30	V_Low	53544.34	25124.46	22022.56		-26.69	-6.24
		Low	52896.99	31046.97	27213.87		-28.24	-24.92
		Mod	78703.98	59042.12	51752.70		13.81	-20.65
		High	69756.41	47306.72	41466.17		2.75	-38.63
	300	V_Low	45600.91	23066.49	20218.67		-48.76	-26.38
		Low	45788.01	31522.24	27630.46		-48.15	-24.90
		Mod	49910.83	15091.99	13228.71		-35.91	-2.08
		High	70628.90	49024.17	42971.58		3.96	17.36

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_d XYL				
			mean	sd	ci	variation to control %	variation to Pristine %
P	0	V_Low	12356.08	6273.11	5498.63		
		Low	14210.98	8724.72	7647.55	13.05	
		Mod	16593.87	9521.63	8346.08	25.54	
		High	15689.82	8888.16	7790.81	21.25	
	3	V_Low	15460.30	7299.22	6398.04	20.08	
		Low	15322.68	12806.46	11225.36	19.36	
		Mod	14719.81	6687.90	5862.20	16.06	
		High	12982.70	3494.51	3063.08	4.83	
	30	V_Low	9465.08	6633.53	5814.54	-30.54	
		Low	10739.22	4845.40	4247.18	-15.06	
		Mod	14719.91	8426.04	7385.75	16.06	
		High	12122.57	4545.30	3984.13	-1.93	
	300	V_Low	11300.36	10200.30	8940.96	-9.34	
		Low	11420.92	7689.61	6740.24	-8.19	
		Mod	10625.79	4920.71	4313.19	-16.28	
		High	10630.62	5630.64	4935.48	-16.23	
			mean	sd	ci		
WWTP	0	V_Low	11740.99	4811.46	4217.43	1.10	-5.24
		Low	11612.21	8188.57	7177.59	0.00	-22.38
		Mod	13494.37	9795.51	8586.14	13.95	-22.97
		High	9888.09	2890.47	2533.60	-17.44	-58.67
	3	V_Low	10435.31	5325.89	4668.35	-11.28	-48.15
		Low	10835.69	10896.16	9550.91	-7.17	-41.41
		Mod	13088.15	5495.22	4816.78	11.28	-12.47
		High	11541.37	4452.47	3902.76	-0.61	-12.49
	30	V_Low	8136.38	9496.93	8324.42	-42.72	-16.33
		Low	7604.63	5529.68	4846.98	-52.70	-41.22
		Mod	9614.77	4694.11	4114.57	-20.77	-53.10
		High	10957.32	4016.31	3520.45	-5.98	-10.63
	300	V_Low	8189.36	2681.35	2350.30	-41.80	-37.99
		Low	8453.92	3870.08	3392.28	-37.36	-35.10
		Mod	9120.43	3710.12	3252.07	-27.32	-16.51
		High	10142.14	6015.99	5273.25	-14.49	-4.82
			mean	sd	ci		
VYRO	0	V_Low	16163.85	12637.07	11076.88	28.28	23.56
		Low	11593.50	8336.91	7307.62	0.00	-22.58
		Mod	15803.70	10352.02	9073.95	26.64	-5.00
		High	16712.03	14851.89	13018.26	30.63	6.12
	3	V_Low	13856.87	9539.33	8361.59	16.33	-11.57
		Low	17499.71	18694.01	16386.02	33.75	12.44
		Mod	15113.90	9317.48	8167.14	23.29	2.61
		High	13862.79	6630.63	5812.00	16.37	6.35
	30	V_Low	8388.71	3702.90	3245.74	-38.20	-12.83
		Low	7966.93	4431.19	3884.11	-45.52	-34.80
		Mod	11977.06	5950.85	5216.15	3.20	-22.90
		High	14049.36	8968.37	7861.13	17.48	13.71
	300	V_Low	9275.92	7583.03	6646.82	-24.98	-21.82
		Low	8095.27	5267.27	4616.96	-43.21	-41.08
		Mod	9955.93	6354.55	5570.01	-16.45	-6.73
		High	13043.10	8181.51	7171.41	11.11	18.50

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_e CEL					
			mean	sd	ci		variation to control %	variation to Pristine %
P	0	V_Low	4519.79	3157.97	2768.09			
		Low	4395.19	3273.93	2869.73		-2.84	
		Mod	4933.27	2803.31	2457.21		8.38	
		High	6493.84	3232.83	2833.70		30.40	
	3	V_Low	4411.81	2824.94	2476.17		-2.45	
		Low	5565.79	4433.59	3886.21		18.79	
		Mod	5393.96	3983.08	3491.32		16.21	
		High	5855.10	1916.57	1679.95		22.81	
	30	V_Low	4968.75	3426.44	3003.41		9.04	
		Low	5350.34	5305.82	4650.75		15.52	
		Mod	4182.27	2782.17	2438.68		-8.07	
		High	6249.57	2773.56	2431.13		27.68	
	300	V_Low	4498.39	4685.02	4106.60		-0.48	
		Low	4367.31	2292.98	2009.89		-3.49	
		Mod	3677.85	2586.10	2266.82		-22.89	
		High	4836.19	1927.29	1689.34		6.54	
			mean	sd	ci			
WWTP	0	V_Low	5035.74	5777.06	5063.81		15.13	10.25
		Low	4274.01	5097.94	4468.54		0.00	-2.84
		Mod	4038.63	2637.78	2312.12		-5.83	-22.15
		High	4852.69	2256.54	1977.95		11.92	-33.82
	3	V_Low	2831.16	1686.69	1478.45		-50.96	-55.83
		Low	4394.70	1856.75	1627.52		2.75	-26.65
		Mod	7111.90	4662.34	4086.73		39.90	24.16
		High	6071.07	2247.50	1970.02		29.60	3.56
	30	V_Low	3749.46	3061.25	2683.31		-13.99	-32.52
		Low	3201.67	2527.51	2215.46		-33.49	-67.11
		Mod	5785.49	4300.30	3769.38		26.13	27.71
		High	7225.08	2797.80	2452.38		40.84	13.50
	300	V_Low	2309.31	915.98	802.89		-85.08	-94.79
		Low	2060.31	825.78	723.83		-107.44	-111.97
		Mod	3531.74	1855.99	1626.84		-21.02	-4.14
		High	3546.10	1866.73	1636.26		-20.53	-36.38
			mean	sd	ci			
VYRO	0	V_Low	4812.30	3949.75	3462.10		15.68	6.08
		Low	4057.81	2365.92	2073.82		0.00	-8.31
		Mod	6382.98	4319.03	3785.80		36.43	22.71
		High	8194.79	5475.09	4799.13		50.48	20.76
	3	V_Low	4720.69	3055.44	2678.21		14.04	6.54
		Low	5289.42	4628.84	4057.36		23.28	-5.22
		Mod	5981.93	4359.57	3821.33		32.17	9.83
		High	6289.44	4042.71	3543.60		35.48	6.91
	30	V_Low	3543.85	1823.70	1598.54		-14.50	-40.21
		Low	2881.35	1665.96	1460.28		-40.83	-85.69
		Mod	4696.12	3088.84	2707.48		13.59	10.94
		High	4210.97	3610.85	3165.05		3.64	-48.41
	300	V_Low	2523.90	1621.99	1421.74		-60.78	-78.23
		Low	3059.89	1142.97	1001.85		-32.61	-42.73
		Mod	4788.62	2900.26	2542.19		15.26	23.20
		High	4396.74	3256.15	2854.14		7.71	-9.99

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_f PHO					
			mean	sd	ci		variation to control %	variation to Pristine %
P	0	V_Low	300633.94	146916.76	128778.21			
		Low	298920.08	107511.35	94237.86		-0.57	
		Mod	336621.46	306575.25	268725.06		10.69	
		High	310507.36	225921.15	198028.62		3.18	
	3	V_Low	258138.45	185097.07	162244.74		-16.46	
		Low	299030.69	218621.74	191630.41		-0.54	
		Mod	267688.22	176993.38	155141.54		-12.31	
		High	244940.04	97149.60	85155.38		-22.74	
	30	V_Low	204116.44	106318.00	93191.84		-47.29	
		Low	352104.99	258807.49	226854.77		14.62	
		Mod	271491.07	149192.51	130773.00		-10.73	
		High	255283.41	169588.17	148650.59		-17.76	
	300	V_Low	347636.66	214770.23	188254.41		13.52	
		Low	211918.26	70653.48	61930.50		-41.86	
		Mod	277084.80	153295.07	134369.05		-8.50	
		High	188857.77	100157.62	87792.03		-59.19	
			mean	sd	ci			
WWTP	0	V_Low	263675.14	105786.55	92726.00		14.17	-14.02
		Low	226319.26	161941.84	141948.29		0.00	-32.08
		Mod	337732.26	338541.72	296744.90		32.99	0.33
		High	158062.68	114804.06	100630.19		-43.18	-96.45
	3	V_Low	264504.16	237629.83	208291.73		14.44	2.41
		Low	348652.92	186981.73	163896.71		35.09	14.23
		Mod	209899.04	122647.37	107505.16		-7.82	-27.53
		High	245478.60	172951.55	151598.72		7.80	0.22
	30	V_Low	304689.87	220023.33	192858.95		25.72	33.01
		Low	299077.92	140591.32	123233.73		24.33	-17.73
		Mod	248967.24	83588.53	73268.58		9.10	-9.05
		High	493321.58	796255.18	697948.43		54.12	48.25
	300	V_Low	201852.91	82098.13	71962.19		-12.12	-72.22
		Low	302039.11	136923.53	120018.77		25.07	29.84
		Mod	227068.60	100501.39	88093.35		0.33	-22.03
		High	249786.38	121495.22	106495.25		9.39	24.39
			mean	sd	ci			
VYRO	0	V_Low	267419.23	128567.30	112694.21		-10.57	-12.42
		Low	295685.56	196814.29	172515.33		0.00	-1.09
		Mod	260956.09	150327.50	131767.86		-13.31	-29.00
		High	415976.19	290961.71	255039.19		28.92	25.35
	3	V_Low	252401.63	172148.54	150894.85		-17.15	-2.27
		Low	294467.58	189001.36	165667.00		-0.41	-1.55
		Mod	409787.82	173741.05	152290.74		27.84	34.68
		High	243043.58	141352.83	123901.22		-21.66	-0.78
	30	V_Low	281357.82	81578.62	71506.81		-5.09	27.45
		Low	223652.73	175209.89	153578.24		-32.21	-57.43
		Mod	270396.72	167777.86	147063.78		-9.35	-0.40
		High	252709.28	231347.11	202784.69		-17.01	-1.02
	300	V_Low	258210.42	111589.46	97812.47		-14.51	-34.63
		Low	238538.24	106614.01	93451.30		-23.96	11.16
		Mod	334082.82	147831.01	129579.60		11.49	17.06
		High	250401.76	265641.93	232845.41		-18.08	24.58

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_h PEP				
			mean	sd	ci	variation to control %	variation to Pristine %
P	0	V_Low	254592.86	339126.92	297257.85		
		Low	83791.56	100914.97	88455.87	-203.84	
		Mod	239215.36	306537.02	268691.54	-6.43	
		High	631732.76	1.17E+06	1022372.46	59.70	
	3	V_Low	166343.89	231206.03	202661.02	-53.05	
		Low	352309.99	518261.59	454276.32	27.74	
		Mod	442396.82	1.22E+06	1070029.59	42.45	
		High	158992.04	342404.70	300130.95	-60.13	
	30	V_Low	148084.77	194907.54	170844.00	-71.92	
		Low	312916.43	697368.47	611270.42	18.64	
		Mod	139190.90	206563.85	181061.20	-82.91	
		High	130587.21	159930.94	140185.65	-94.96	
	300	V_Low	156452.29	321402.44	281721.66	-62.73	
		Low	125815.85	175058.22	153445.30	-102.35	
		Mod	69862.32	98187.45	86065.10	-264.42	
		High	70140.83	105056.79	92086.34	-262.97	
			mean	sd	ci		
WWTP	0	V_Low	174823.06	230948.07	202434.91	-93.22	-45.63
		Low	337785.06	490801.42	430206.42	0.00	75.19
		Mod	308602.13	318389.14	279080.39	-9.46	22.48
		High	294293.84	628459.86	550869.36	-14.78	-114.66
	3	V_Low	197086.63	229391.11	201070.18	-71.39	15.60
		Low	114203.18	266738.21	233806.35	-195.78	-208.49
		Mod	765786.99	2.18E+06	1911558.60	55.89	42.23
		High	182570.29	165078.05	144697.29	-85.02	12.91
	30	V_Low	135279.96	187911.43	164711.63	-149.69	-9.47
		Low	76215.85	89720.73	78643.69	-343.20	-310.57
		Mod	107894.81	152439.96	133619.52	-213.07	-29.01
		High	120039.34	140237.34	122923.45	-181.40	-8.79
	300	V_Low	88240.47	106796.11	93610.92	-282.80	-77.30
		Low	96922.22	116605.94	102209.61	-248.51	-29.81
		Mod	305974.98	616829.70	540675.07	-10.40	77.17
		High	57171.27	74183.81	65024.97	-490.83	-22.69
			mean	sd	ci		
VYRO	0	V_Low	202487.36	400266.12	350848.73	-63.16	-25.73
		Low	330379.03	992794.04	870222.34	0.00	74.64
		Mod	213230.14	177267.40	155381.73	-54.94	-12.19
		High	263786.38	387197.73	339393.78	-25.24	-139.49
	3	V_Low	354409.13	831560.41	728894.84	6.78	53.06
		Low	192452.83	323046.50	283162.74	-71.67	-83.06
		Mod	352653.74	576298.13	505147.58	6.32	-25.45
		High	257234.83	326240.67	285962.56	-28.43	38.19
	30	V_Low	137331.46	243277.34	213241.99	-140.57	-7.83
		Low	172119.48	281860.39	247061.52	-91.95	-81.80
		Mod	77538.72	92119.48	80746.28	-326.08	-79.51
		High	106615.83	92850.08	81386.68	-209.88	-22.48
	300	V_Low	116821.76	215222.44	188650.79	-182.81	-33.92
		Low	210620.26	224880.21	197116.20	-56.86	40.26
		Mod	200505.50	302811.51	265425.99	-64.77	65.16
		High	226911.00	248874.30	218147.95	-45.60	69.09

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_i PHE					
			mean	sd	ci		variation to control %	variation to Pristine %
P	0	V_Low	370.38	206.44	180.95			
		Low	242.71	134.34	117.75		-52.60	
		Mod	405.50	341.09	298.98		8.66	
		High	380.41	263.77	231.20		2.64	
	3	V_Low	476.19	297.43	260.71		22.22	
		Low	292.94	358.03	313.82		-26.43	
		Mod	373.51	314.83	275.96		0.84	
		High	410.41	246.22	215.82		9.75	
	30	V_Low	301.74	262.15	229.78		-22.75	
		Low	701.63	932.78	817.62		47.21	
		Mod	422.36	349.50	306.35		12.31	
		High	521.28	525.98	461.04		28.95	
	300	V_Low	420.25	471.44	413.23		11.87	
		Low	277.73	250.92	219.94		-33.36	
		Mod	274.19	145.71	127.72		-35.08	
		High	247.83	184.13	161.40		-49.45	
			mean	sd	ci			
WWTP	0	V_Low	259.52	161.07	141.18		-18.06	-42.72
		Low	306.37	225.76	197.89		0.00	20.78
		Mod	409.05	320.29	280.75		25.10	0.87
		High	370.33	227.44	199.36		17.27	-2.72
	3	V_Low	472.54	382.76	335.51		35.17	-0.77
		Low	344.51	229.80	201.43		11.07	14.97
		Mod	236.11	202.43	177.44		-29.76	-58.19
		High	217.98	115.99	101.67		-40.55	-88.28
	30	V_Low	356.33	602.49	528.11		14.02	15.32
		Low	395.98	685.37	600.75		22.63	-77.19
		Mod	527.82	711.84	623.96		41.95	19.98
		High	392.29	415.39	364.11		21.90	-32.88
	300	V_Low	215.53	351.17	307.82		-42.15	-94.98
		Low	221.14	153.41	134.47		-38.54	-25.59
		Mod	219.90	146.42	128.34		-39.33	-24.69
		High	262.27	268.85	235.66		-16.82	5.50
		mean	sd	ci	0.00			
VYRO	0	V_Low	310.17	221.32	193.99		-2.88	-19.41
		Low	319.09	206.77	181.24		0.00	23.94
		Mod	551.00	330.64	289.82		42.09	26.41
		High	346.64	185.02	162.17		7.95	-9.74
	3	V_Low	438.67	310.96	272.57		27.26	-8.55
		Low	337.04	303.93	266.41		5.32	13.08
		Mod	445.46	204.27	179.05		28.37	16.15
		High	238.70	158.34	138.79		-33.68	-71.93
	30	V_Low	379.77	356.82	312.77		15.98	20.55
		Low	258.68	214.58	188.09		-23.36	-171.24
		Mod	442.49	429.87	376.80		27.89	4.55
		High	287.64	245.55	215.24		-10.93	-81.22
	300	V_Low	309.20	241.73	211.89		-3.20	-35.92
		Low	254.60	202.10	177.15		-25.33	-9.09
		Mod	282.95	144.75	126.88		-12.77	3.10
		High	419.09	310.26	271.95		23.86	40.86

Table S10 - Mean of each endpoint evaluated, sd- standard error, ci – 95% confidence intervals; and respective variation to control and to Pristine – continuation.

Land-use History	Total fungicide concentration ug/L	NO3-N levels	Sub_k PER					
			mean	sd	ci		to control %	to Pristine %
P	0	V_Low	252.55	185.87	162.92			
		Low	224.59	123.50	108.26		-12.45	
		Mod	288.95	326.13	285.86		12.60	
		High	317.08	190.09	166.62		20.35	
	3	V_Low	204.33	199.05	174.47		-23.59	
		Low	260.95	152.87	134.00		3.22	
		Mod	314.81	252.95	221.72		19.78	
		High	258.25	160.73	140.89		2.21	
	30	V_Low	216.29	124.34	108.99		-16.76	
		Low	339.41	377.98	331.31		25.59	
		Mod	253.79	125.56	110.06		0.49	
		High	255.44	135.41	118.69		1.13	
	300	V_Low	249.74	217.74	190.86		-1.12	
		Low	260.02	251.27	220.25		2.87	
		Mod	242.39	169.59	148.65		-4.19	
		High	273.16	197.45	173.07		7.55	
			mean	sd	ci			
WWTP	0	V_Low	213.13	152.10	133.33		14.37	-18.49
		Low	182.49	128.92	113.00		0.00	-23.07
		Mod	213.32	175.38	153.73		14.45	-35.45
		High	269.03	224.47	196.76		32.17	-17.86
	3	V_Low	177.45	168.53	147.72		-2.84	-15.15
		Low	150.40	120.10	105.28		-21.34	-73.51
		Mod	175.56	126.75	111.10		-3.95	-79.32
		High	208.87	71.99	63.11		12.63	-23.64
	30	V_Low	205.99	251.87	220.77		11.41	-5.00
		Low	208.13	74.98	65.73		12.32	-63.08
		Mod	155.61	98.44	86.29		-17.27	-63.09
		High	237.77	158.34	138.79		23.25	-7.43
	300	V_Low	146.21	158.39	138.84		-24.82	-70.81
		Low	174.65	117.63	103.11		-4.49	-48.88
		Mod	138.32	107.91	94.58		-31.94	-75.24
		High	190.73	186.87	163.80		4.32	-43.22
			mean	sd	ci			
VYRO	0	V_Low	255.99	120.78	105.87		25.40	1.34
		Low	190.98	177.58	155.65		0.00	-17.60
		Mod	256.28	221.19	193.88		25.48	-12.75
		High	303.59	193.77	169.84		37.09	-4.44
	3	V_Low	246.52	208.46	182.72		22.53	17.11
		Low	195.55	170.17	149.16		2.34	-33.44
		Mod	292.79	265.74	232.93		34.77	-7.52
		High	265.47	242.83	212.85		28.06	2.72
	30	V_Low	244.03	256.77	225.07		21.74	11.37
		Low	150.49	185.10	162.25		-26.90	-125.54
		Mod	250.73	193.67	169.76		23.83	-1.22
		High	234.96	193.86	169.92		18.72	-8.71
	300	V_Low	282.75	264.34	231.70		32.46	11.67
		Low	238.81	263.94	231.36		20.03	-8.88
		Mod	215.82	174.65	153.09		11.51	-12.31
		High	282.51	215.85	189.20		32.40	3.31

Table S11 - Post-hoc testing of aligned ranks transformation ANOVA, for leaf decomposition, bacterial and fungal operon copies as proxies for their abundance. Df, degrees of freedom; ratio of variances; SE, standard error of the estimate. P: pristine; W: wastewater V: vineyard run-off.

Endpoint						
Leaf litter decomposition	Post-hoc - Nutrient	estimate	SE	df	t.ratio	p.value
	Vlow-Low	-81.8	18.6	592	-4.394	0.0001
	Vlow-Mod	-179.2	18.6	592	-9.623	<.0001
	Vlo-High	-253.6	18.6	592	-13.616	<.0001
	Low-Mod	-97.4	18.6	592	-5.229	<.0001
	Low-High	-171.8	18.6	592	-9.222	<.0001
	Mod-high	-74.4	18.6	592	-3.993	0.0004
	Post-hoc - History	estimate	SE	df	t.ratio	p.value
	P - V	-54.7	19.3	592	-2.829	0.0145
	P - W	-57.1	17.3	592	-3.301	0.0031
	V - W	-2.4	19.3	592	-0.124	1
Bacteria abundance	Post-hoc - Fungicide	estimate	SE	df	t.ratio	p.value
	0 - 3	-13.3	15	336	-0.884	1
	0 - 30	-33.1	15	336	-2.207	0.1681
	0 - 300	38.9	15	336	2.592	0.0598
	3 - 30	-19.8	15	336	-1.323	1
	3 - 300	52.2	15	336	3.476	0.0035
	30 - 300	72	15	336	4.799	<.0001
	Post-hoc - History	estimate	SE	df	t.ratio	p.value
	P - V	-3.08	13.8	336	-0.223	1
	P - W	29.9	12.3	336	2.425	0.0475
	V - W	32.98	13.8	336	2.392	0.0519
Fungi abundance	Post-hoc - Fungicide	estimate	SE	df	t.ratio	p.value
	0 - 3	-10.3	14.2	336	-0.725	1
	0 - 30	-40.7	14.2	336	-2.857	0.0272
	0 - 300	26	14.2	336	1.825	0.413
	3 - 30	-30.4	14.2	336	-2.132	0.2023
	3 - 300	36.3	14.2	336	2.55	0.0672
	30 - 300	66.7	14.2	336	4.682	<.0001

Table S12 -Means ± sd of fungal and bacterial operon copies (10^8/mg leaf dry weight; n=3) as a proxy for abundances, of microbial communities colonizing alder leaves after fungicide and nutrient exposure.

Community history		Pristine				Wastewater				Vineyard			
Total fungicide concentration µg/L	Nutrients levels	Bacterial DNA copies (10^8 operon copies/mg leaf dw)		Fungal DNA copies (10^8 operon copies/mg leaf dw)		Bacterial DNA copies (10^8 operon copies/mg leaf dw)		Fungal DNA copies (10^8 operon copies/mg leaf dw)		Bacterial DNA copies (10^8 operon copies/mg leaf dw)		Fungal DNA copies (10^8 operon copies/mg leaf dw)	
		mean	±sd	mean	±sd	mean	±sd	mean	±sd	mean	±sd	mean	±sd
0	V_Low	5.05	2.32	33.80	11.70	20.70	24.80	96.20	102.00	4.79	4.60	30.90	27.70
	Low	36.50	49.00	151.00	192.00	6.00	1.12	40.30	5.82	1.66	1.17	13.60	9.68
	Mod	5.67	2.06	40.70	14.50	13.50	9.64	71.90	34.70	3.06	2.32	21.80	15.80
	High	7.83	1.37	52.10	5.47	28.70	17.40	130.00	62.90	2.95	2.11	22.10	15.70
3	V_Low	11.20	2.97	64.70	10.80	4.10	1.58	28.20	7.90	3.43	3.84	21.00	22.70
	Low	10.90	1.28	69.70	0.87	3.80	1.13	28.50	7.30	1.97	2.10	14.80	15.20
	Mod	15.20	12.50	82.90	58.40	8.65	0.25	56.20	2.31	9.21	11.90	48.10	58.00
	High	4.52	0.34	32.40	1.63	22.00	10.10	123.00	51.80	2.13	2.19	16.10	15.30
30	V_Low	16.90	9.27	90.80	43.10	9.05	7.33	53.10	35.50	1.85	1.53	14.60	11.30
	Low	4.38	1.86	31.50	11.50	4.47	0.35	32.30	3.96	2.71	2.19	19.30	15.10
	Mod	4.76	0.16	34.50	1.11	5.68	0.88	39.80	6.84	4.10	3.34	27.40	20.90
	High	4.44	0.46	32.40	3.65	7.32	2.58	49.30	14.90	2.69	2.82	18.00	16.80
300	V_Low	12.10	12.40	59.70	49.20	2.87	0.22	21.90	2.33	2.43	2.46	17.50	16.80
	Low	4.93	3.64	31.20	17.70	12.10	13.90	58.40	57.40	3.03	2.71	20.00	16.00
	Mod	5.41	3.01	36.20	15.40	4.80	1.57	32.20	8.17	1.59	1.27	12.30	9.27
	High	2.74	1.33	21.30	8.94	6.80	5.96	41.80	30.70	0.92	0.69	8.15	5.99

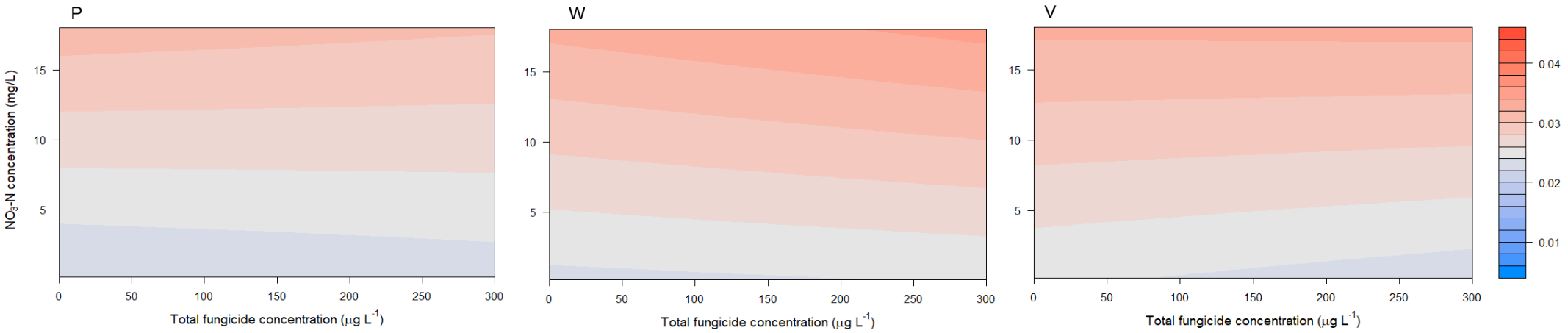

Figure S1. Two-dimensional surface plots displaying the microbial leaf litter decomposition rate ($k_{microbial}$ (d^{-1}); n = 5 for each tested combination of fungicides and nutrients) observed for the each of the community history categories against a surface defined by the total fungicide concentration and the NO_3-N concentration (as one representative for the nutrient treatment). P: pristine; W:wastewater V: vineyard run-off

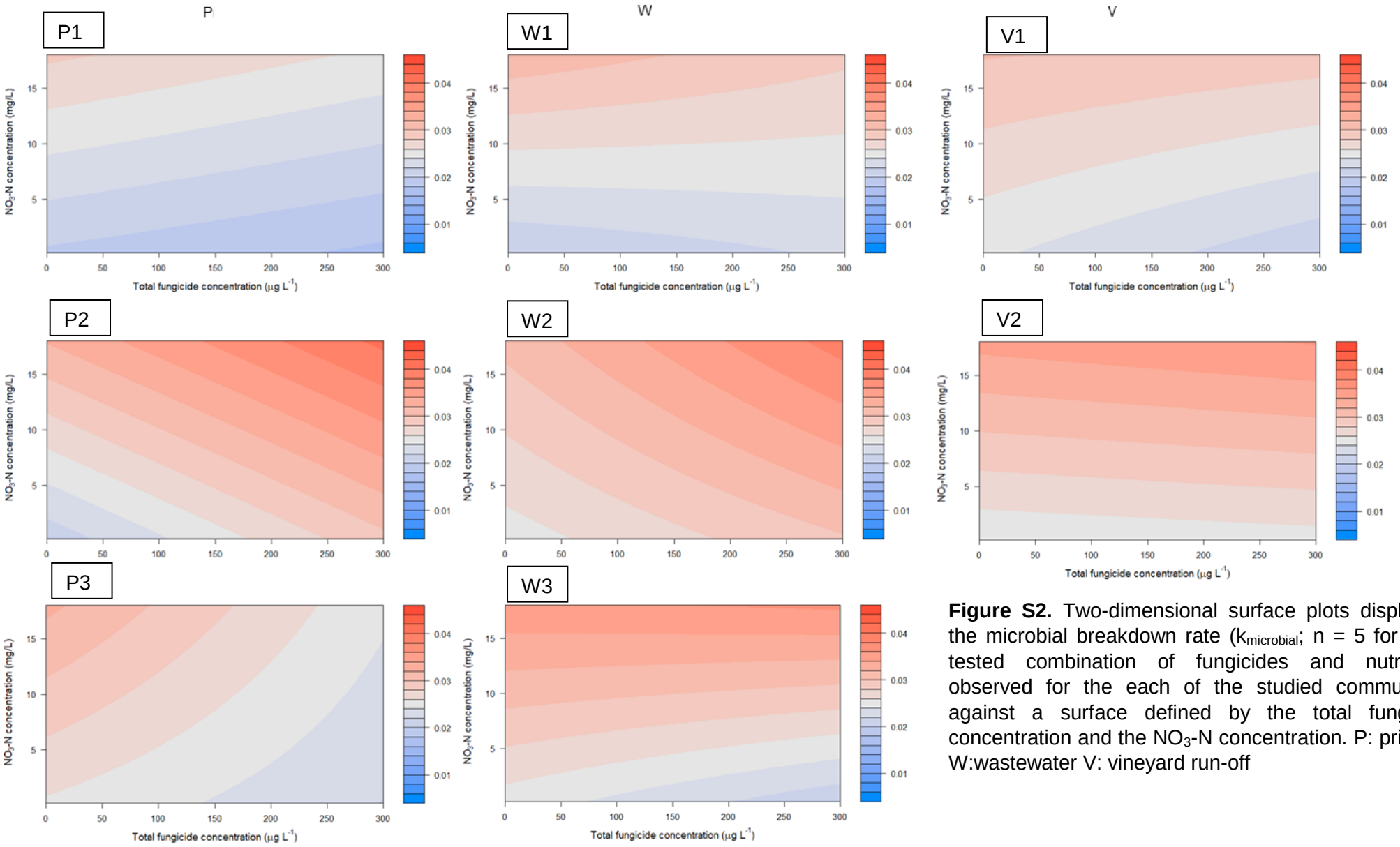

Figure S2. Two-dimensional surface plots displaying the microbial breakdown rate ($k_{microbial}$; n = 5 for each tested combination of fungicides and nutrients) observed for the each of the studied communities against a surface defined by the total fungicide concentration and the NO_3-N concentration. P: pristine; W:wastewater V: vineyard run-off

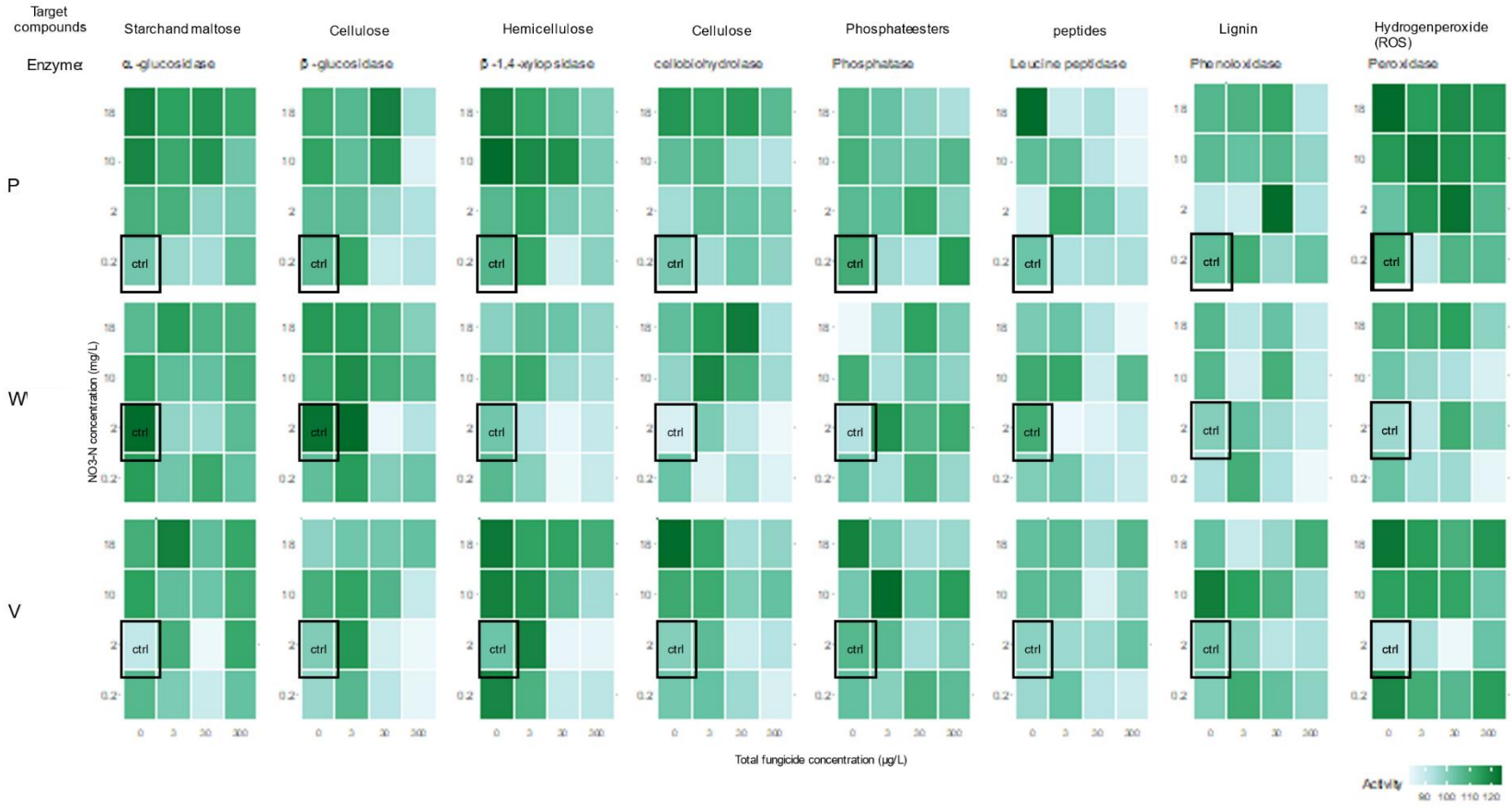

Figure S3. Heatmaps displaying square root-transformed activities, in µmol of degraded substrate/g leaf dry mass/hour, of β-1,4-glucosidase (BGL; targeting cellulose), β-1,4-xylosidase (XYL; targeting hemicellulose), cellobiohydrolase (CEL; targeting cellulose), phosphatase (PHO; targeting phosphate esters), phenol oxidase (PHE; targeting lignin) and peroxidase (PER; targeting lignin). Leaf species are shown on the Y-axis, while the community histories are shown on the x-axis (P: pristine; W:wastewater V: vineyard run-off).

Figure S4. Heatmaps displaying square root-transformed activities, in µmol of degraded substrate/g leaf dry mass/hour, of β-1,4-glucosidase (BGL; targeting cellulose), β-1,4-xylosidase (XYL; targeting hemicellulose), cellobiohydrolase (CEL; targeting cellulose), phosphatase (PHO; targeting phosphate esters), phenol oxidase (PHE; targeting lignin) and peroxidase (PER; targeting lignin). Leaf species are shown on the Y-axis, while the community histories are shown on the x-axis (P: pristine; W: wastewater treatment plant; V: vineyard).

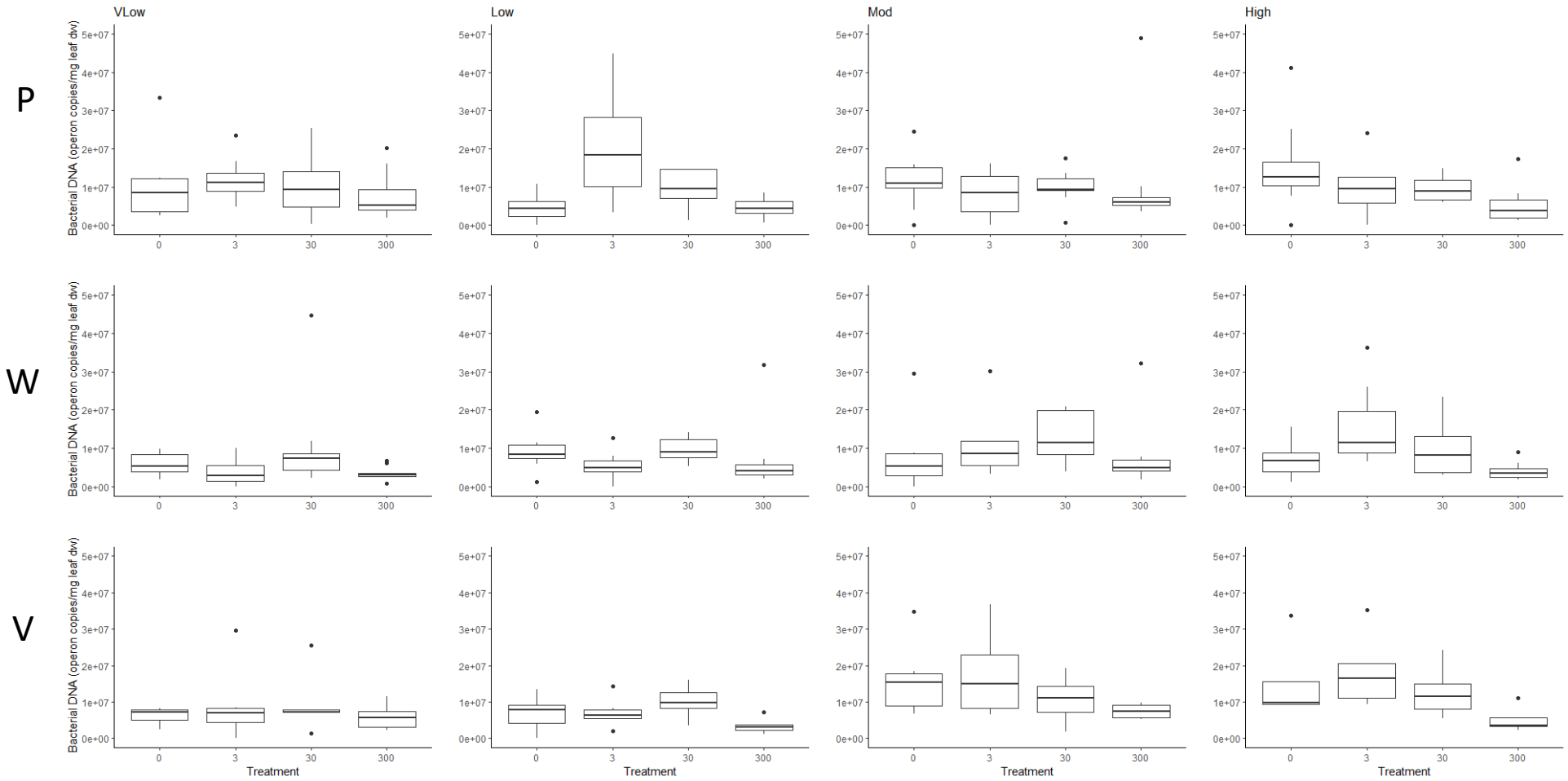

Figure S5. Bacterial operon copy number (n = 3) as a proxy for abundance for each tested combination of fungicides and nutrients (mean values ± standard deviation, n = 3). P - Pristine; W- wastewater; V- vineyard.

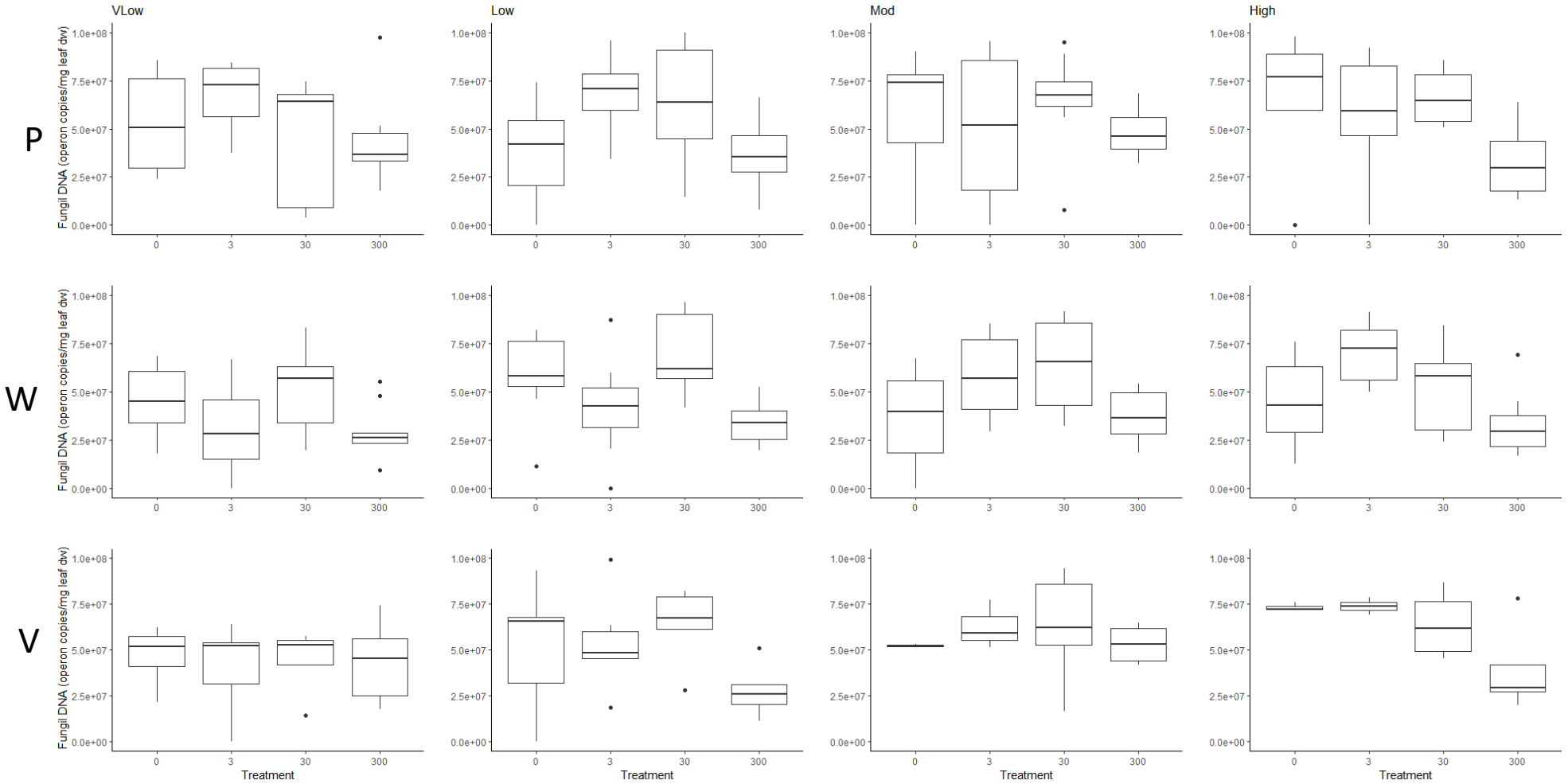

Figure S6. Fungal operon copy number (n = 3) as a proxy for abundance for each tested combination of fungicides and nutrients (mean values ± standard deviation, n = 3). P - Pristine; W- wastewater; V- vineyard.

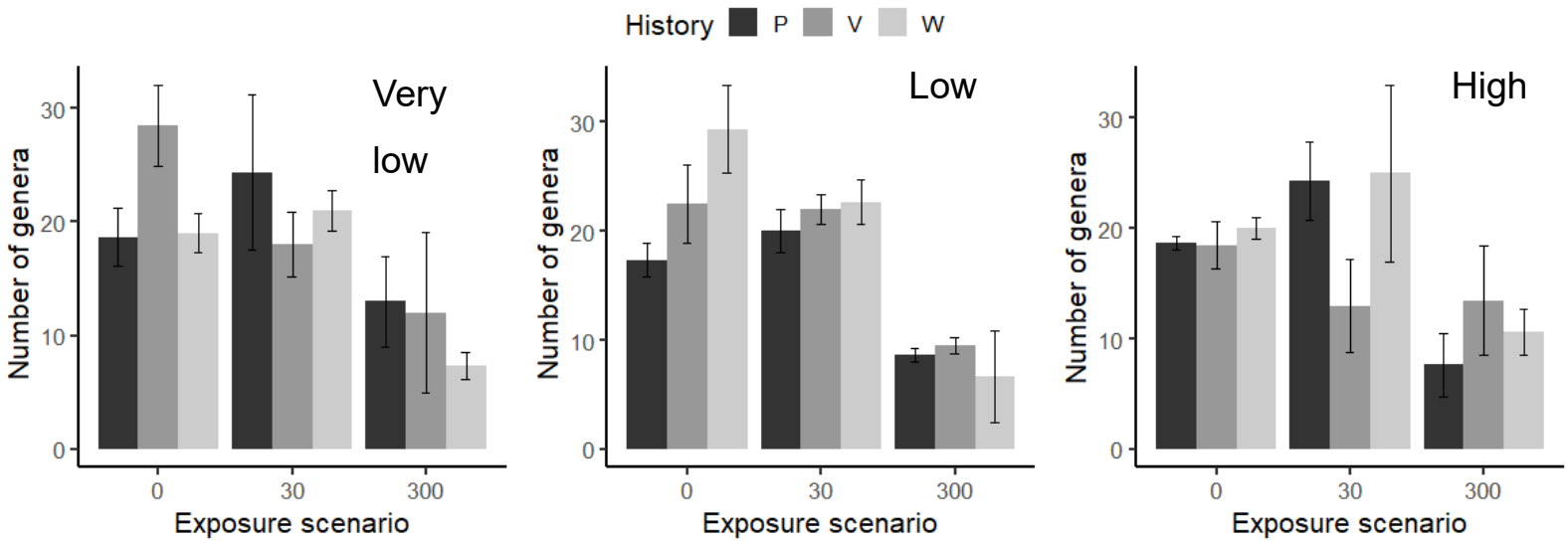

Figure S7. Number of curated genera for each tested combination of fungicides and nutrients (mean values ± standard deviation, n = 3). P - Pristine; W- wastewater; V- vineyard.

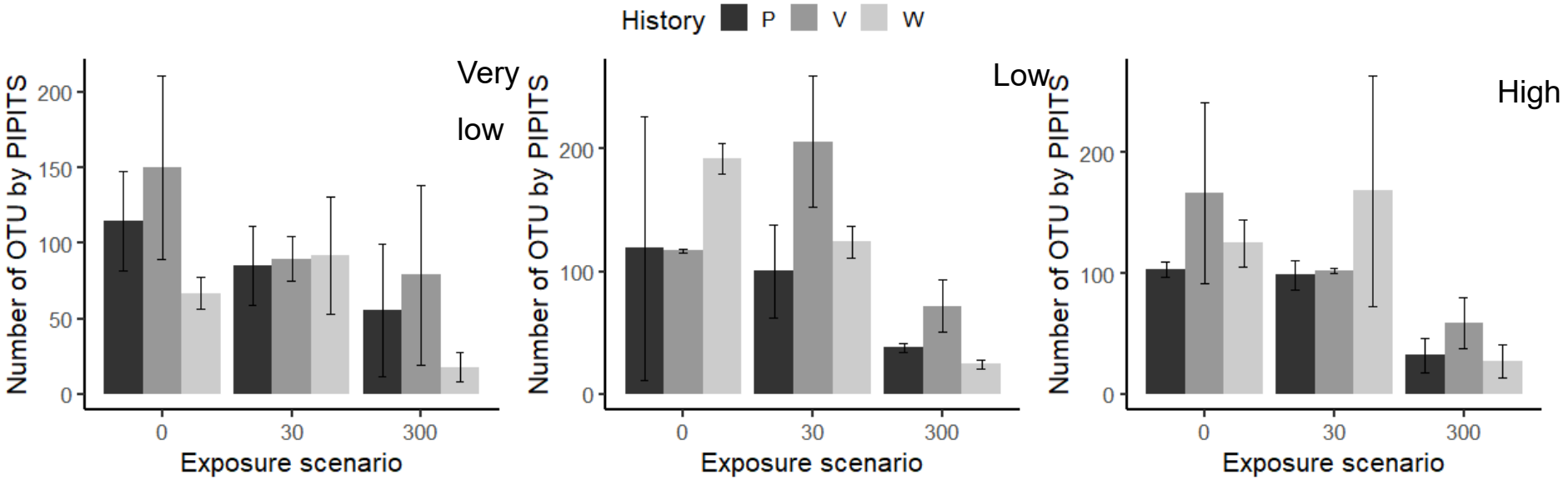

Figure S8. Number of curated OTUs (Operational taxonomic units) as a proxy for taxa richness for each tested combination of fungicides and nutrients (mean values ± standard deviation, n = 3). P - Pristine; W- wastewater; V- vineyard.

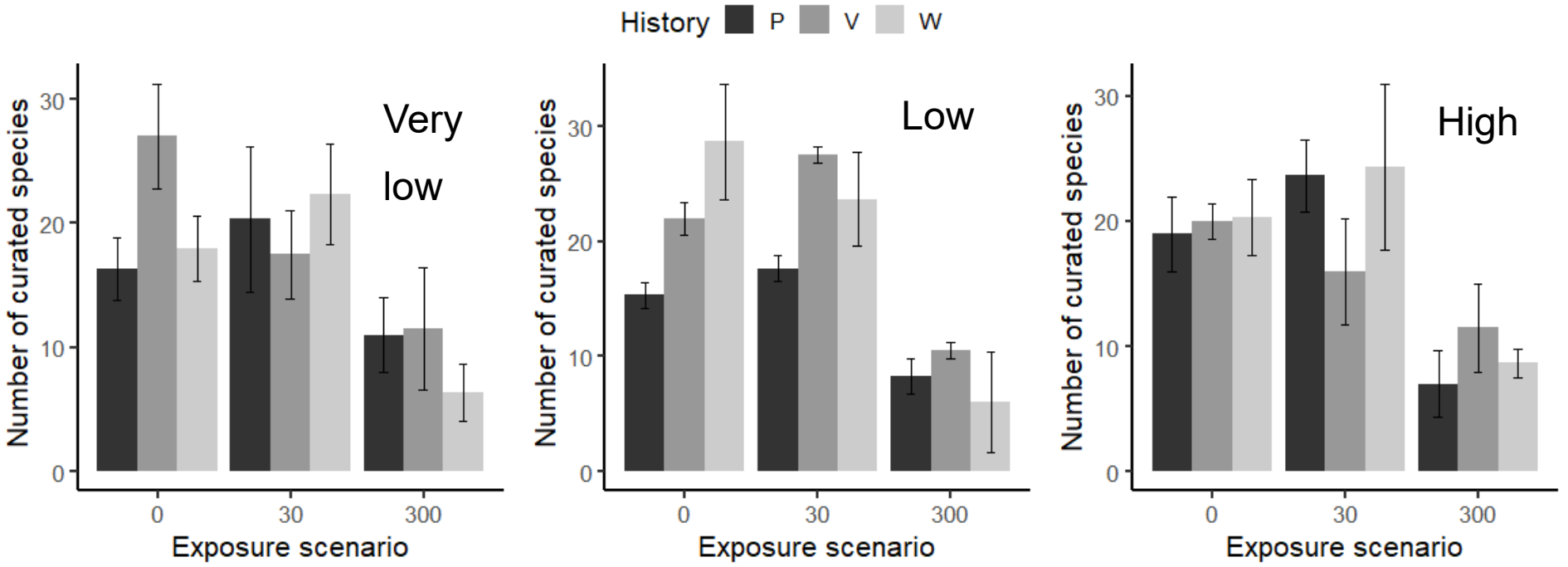

Figure S9. Number of curated species via Genbank (ncbi) for each tested combination of fungicides and nutrients (mean values ± standard deviation, n = 3). P - Pristine; W- wastewater; V- vineyard.

A.2 Material and methods

A.2.1 Protocol for fungicide measurements according to Fernández et al., 2014

A subsample of thawed medium was taken after vortexed. These subsamples were centrifuged at 4000 rpm for 30 minutes and the supernatants were used further chemical analysis. A ratio of 10% methanol was used to extract samples and standards (PESTANAL from Sigma-Aldrich). Exactive (LC-HRMS) Orbitrap system (Thermo Fisher Scientific Corporation) was used to measure both samples and standards. While 50 x 2.1 mm Thermo Hypersil GOLD™ column (1.9 mm particle size) was used for fungicide separation, in this study the mobile phase used was H_2O/MeOH with 0.1% formic acid (without 4 mM NH4 formate). The injection volume used was 20 µg/L and the calibration curve matrix matched with used medium. More detailed information can be found in Fernández, D., Vermeirssen, E.L.M., Bandow, N., Muñoz, K., Schäfer, R.B., 2014. Calibration and field application of passive sampling for episodic exposure to polar organic pesticides in streams. Environmental Pollution 194, 196–202. https://doi.org/10.1016/j.envpol.2014.08.001.

A.2.2 Exoenzyme activity

To quantify hydrolases and oxidases activities, we use the method described by DeForest (2009) but modified for leaf litter (see Baudy et al. 2021). Hydrolases, namely β-1,4-glucosidase (BGL; EC 3.2.1.21; targeting cellulose), cellobiohydrolase (CEL; EC 3.2.1.91; targeting cellulose), β-1,4-xylosidase (XYL; EC 3.2.1.37; targeting hemicellulose), and phosphatases (PHO; EC 3.1.3.1 and 3.1.3.2; targeting phosphate esters), were measured fluorometrically using fluorescent (MUF, methylumbelliferone)-linked artificial substrates. Oxidases, namely phenol oxidase (PHE; EC 1.10.3.2; targeting lignin) and peroxidase (PER; EC 1.11.1.7; targeting lignin), were measured colorimetrically employing L-3,4-dihydroxyphenylalanine (L-DOPA).

After thawing, 1 leaf disc (2 cm diameter) was homogenized in 350 mL of nutrient medium using an Ultra-turrax® blender (IKA®-Werke GmbH and Co. KG, Germany) at 24,000 rpm. For hydrolase analyses, black flat-bottom 96-well 300-µL plates (Thermo Fisher Scientific, USA) were incubated in darkness for 1 h on a rotary shaker (model KS 15; Edmund Bühler GmbH, Germany) at 120 rpm, whereupon 10 µL 1M NaOH were added to terminate reactions and enhance fluorescence (DeForest 2009). Fluorescence was measured at 365 nm excitation and 450 nm emission using a microplate reader (Infinite 200, Tecan Group; Switzerland). Oxidases were measured in clear flat-bottom 96-well 300-µL plates (Thermo Fisher Scientific, USA), after incubation for 2 h on a rotary shaker. Absorbance was measured at 450 nm using a microplate reader. The medium containing the homogenized leaves was filtered through pre-weighed glass fibre filters (GF/6, Whatman, Dassel, Germany) and dried at 60 ºC for 24 h to

determine leaf dry mass to the nearest 0.01 mg. Enzymatic activity was expressed as µmol of degraded substrate/g leaf dry mass/hour (DeForest 2009). Further details on substrate concentrations, plate layout and calculations can be found in Baudy et al. (2021).

DeForest, J. L. (2009). The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. Soil Biology and Biochemistry, 41(6), 1180–1186. https://doi.org/10.1016/j.soilbio.2009.02.029

Baudy, P., Zubrod, J. P., Konschak, M., Kolbenschlag, S., Pollitt, A., Baschien, C., & Schulz, R. (2021). Fungal – fungal and fungal – bacterial interactions in aquatic decomposer communities: bacteria promote fungal diversity, 102(November 2020), 1–16. https://doi.org/10.1002/ecy.3471

A.2.3 Next generation sequencing - Protocol from Carl et al. 2022

Preparation of leaf samples for sequencing on the Illumina MiSeq platform included DNA extraction and a 3-step-PCR with DNA extracts (Lindahl et al., 2013), followed by clean-up, DNA concentration measurements, equalization, and pooling of the resulting PCR products. Total DNA was extracted using the FastDNA SPIN Kit for Soil and the FastPrep-24 instrument (MP Biomedicals, Solon, USA. Further extraction steps were performed according to the manufacturer's protocol including the recommendations of extended time for debris centrifugation (15 min), protein precipitation on ice, and incubation of resuspended binding matrix for 5 min at 55 ºC and 550 rpm before elution of DNA in 75 µLµL of the supplied PCR grade water. DNA extracts were stored at 4 ºC until needed. Amplicon libraries of the fungal ITS2 rDNA gene were generated using a mix of five forward primers ('ITS3tagmix') and one reverse primer ('ITS4ngs'), which address more than 95% of the known fungal kingdom (Tedersoo et al., 2014, 2015). PCR was conducted using the Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, USA) in 20 µL reactions with 12.2 µL water (PCR grade), 4 µL 5× HF buffer (supplied with DNA polymerase, 1 mM final MgCl2 concentration), 1.6 µL dNTPs (Thermo Fisher Scientific, 2.5 mM each) and 0.5 µL each of 20 pM forward primer mix (5 primer with 4 pM each) and reverse primer. PCR was run on a thermal cycler (Bio-rad C100 touch, Hercules, USA) with 30 s initial denaturation at 98ºC, 30 amplification cycles of 10 s at 98 ºC, 30 s primer annealing at 55 ºC, and 1 min elongation at 72 ºC followed by a final elongation at 72 ºC for 10 min. All DNA-extracts were diluted 100-fold using the PCR grade water from the extraction kit in order to reduce the influence of PCR inhibitors and to avoid further clean-up steps that might lead to the loss of DNA. All diluted DNA extracts were amplified twice and the PCR products were pooled for each sample to account for the technical bias of PCR reactions (Lindahl et al., 2013). For metabarcoding, two more PCRs were performed, where barcodes, sequencing adaptors, and indices were ligated to the products of the first PCR. To achieve a distinct sample assignment of sequences, samples were grouped into 9 indices with 9 barcodes. To prevent cross-contamination of different treatments by potential barcode hoppers (Nilsson et al., 2019), samples of the same

treatment were ligated with one index only. PCR products were always stored at 4 ºC until further processing and the amplification success for all reactions was checked via electrophoresis on 1% agarose gels for products of the first and second PCR (pre-amplification and barcoding), or 1.7% agarose gels for index PCR products, respectively. After barcoding and indexing, the resulting index PCR products were purified with innuPREP PCR pure Kit (Analytik Jena, Jena, Germany) and their DNA concentration was quantified using the Quant-iT PicoGreen dsDNA assay (Invitrogen, Carlsbad, USA). All PCR products were then diluted with PCR grade water to a final concentration of 4 nM, before they were pooled in a 1.5 ml tube (4 µL per sample). The resulting ITS2 library was then sequenced on the Illumina MiSeq System at a concentration of 4.4 pM with a 0.6 pM addition of an Illumina generated PhiX control library using the chemistry of a 600-cycle MiSeq Reagent Kit v3 (Illumina, San Diego, USA). PairPaired-end sequencing generated 2 × 300 bp reads. Demultiplexing of indices was performed automatically in the MiSeq sequencer according to a predefined sample sheet including the index sequences, whereas barcodes were demultiplexed using an in-house script of the Leibniz Institute DSMZ (https://github.com/boykebunk/ amplicon). Subsequently, sequences were processed with PIPITS (Version 2.4, Gweon et al., 2015, https://github.com/hsgweon/pipits /releases), an automated pipeline, which was especially recommended for Illumina derived sequences (Anslan et al., 2018; Nilsson et al., 2019). PIPITS includes sequence quality filtering with fastx, extraction of ITS subregions with ITSx, chimera filtering according to the UNITE UCHIME database, as well as clustering of OTUs with VSEARCH. Thus, ITS2 sequences were extracted from raw reads with relaxed threshold values for removal of flanking genes (Bengtsson-Palme et al., 2013). An ITS sequence similarity threshold of 97% was used for the generation of operational taxonomic units (OTUs). Taxonomic assignment was performed using the trained datasets of the RDP classifier (UNITE DB version February 02, 2019). In this way, PIPITS created an OTU table for every sample, which was assigned according to the ‘Species Hypothesis’ (SH) of the UNITE database (Nilsson et al., 2018).

Carl, S., Mohr, S., Sahm, R., & Baschien, C. (2022). Laboratory conditions can change the complexity and composition of the natural aquatic mycobiome on Alnus glutinosa leaf litter. Fungal Ecology, 57–58. https://doi.org/10.1016/J.FUNECO.2022.101142

Orn, B., Lindahl, D., Nilsson, R. H., Tedersoo, L., Abarenkov, K., Carlsen, T., … Kauserud, H. (2013). Methods Fungal community analysis by high-throughput sequencing of amplified markers – a user’s guide. New Phytologist, 199(2004), 288–299. https://doi.org/10.1111/nph.12243

Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., … Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research, 47(D1), D259–D264. https://doi.org/10.1093/NAR/GKY1022

Gweon, H. S., Oliver, A., Taylor, J., Booth, T., Gibbs, M., Read, D. S., … Schonrogge, K. (2015). PIPITS: an automated pipeline for analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. Methods in Ecology and Evolution, 6(8), 973–980. https://doi.org/10.1111/2041-210X.12399

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., …Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS 2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4 (10), 914-919

7.3 APPENDIX III

Microbial community history and leaf species shape bottom-up effects in a freshwater shredding amphipod

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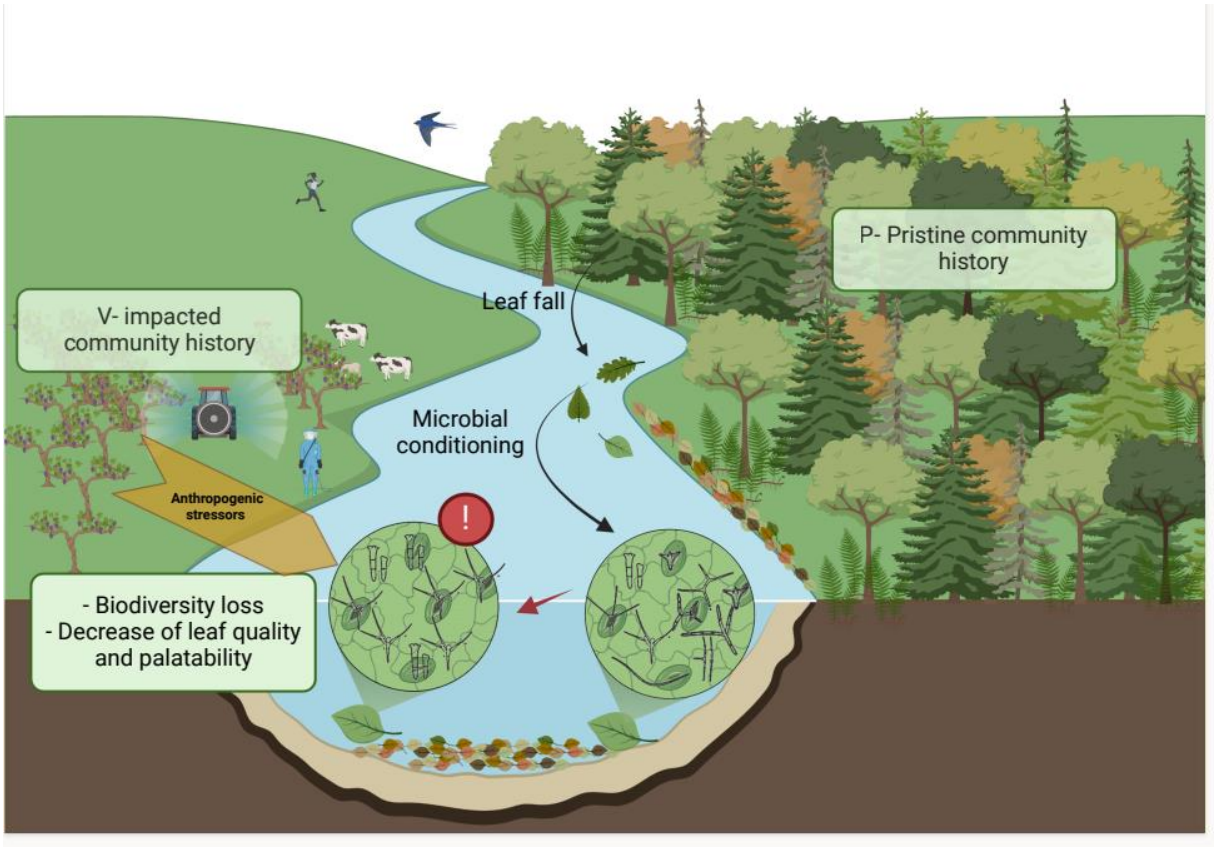
ABSTRACT

Arable land use and the associated application of agrochemicals can affect local freshwater communities with consequences for the entire ecosystem. For instance, the structure and function of leaf-associated microbial communities can be affected by pesticides, such as fungicides. Additionally, the leaf species on which these microbial communities grow reflects another environmental filter for community structure. These factors and their interaction may jointly modify leaves' nutritional quality for higher trophic levels. To test this assumption, we studied the structure of leaf-associated microbial communities with distinct exposure histories (pristine [P] vs vineyard run off [V]) colonising two leaf species (black alder, European beech, and a mixture thereof). By offering these differently colonised leaves as food to male and female individual of the leaf-shredding amphipod *Gammarus fossarum* (Crustacea; Amphipoda) we assessed for potential bottom-up effects. The growth rate, feeding rate, faeces production and neutral lipid fatty acid profile of the amphipod served as response variable in a 2x2x3-factorial test design over 21d. A clear separation of community history (P vs V), leaf species and an interaction between the two factors was observed for the leaf-associated aquatic hyphomycete (i.e., fungal) community. Sensitive fungal species were reduced by up to 70% in V- compared to P-communities. *Gammarus'* growth rate, feeding rate and faeces production were affected by the factor leaf species. Growth was negatively affected when *Gammarus* were fed with beech leaves only, whereas the impact of alder and the mixture of both leaf species was sex-specific. Overall, this study case highlights that leaf species identity had a more substantial impact on gammarids relative to the microbial community itself. Furthermore, the sex-specificity of the observed effects (excluding lipid fatty acid, profile which was only measured for male) questions the procedure of earlier studies, that is using either only one sex or not being able to differentiate between males and females. However, these results need additional verification to support a reliable extrapolation.

Keywords: Leaf litter breakdown, Shredders, Aquatic fungi, Exposure history, Food quality, Fatty acids

GRAPHICAL ABSTRACT



Created with BioRender.com

INTRODUCTION

The decomposition of allochthonous organic carbon, such as terrestrial leaf litter, is a fundamental ecosystem-level process in streams with forest-dominated catchments (Fisher & Likens, 1973; Minshall, 1967; Nelson & Scott, 1962). After leaching of soluble organic substances, leaf litter is colonised by aquatic microorganisms, such as aquatic hyphomycetes (AH; a polyphyletic group of asexual fungi; Baschien, Marvanová & Szewzyk, 2006; Ferreira et al., 2016) and bacteria (Gessner, Chauvet & Dobson, 1999). These microorganisms decompose leaf litter by producing exoenzymes responsible for the transformation of complex leaf compounds into more usable and accessible transformation products (Hieber & Gessner, 2002). Moreover, the activity of bacteria and fungi increases the leaves' palatability and nutritional value for leaf-shredding invertebrates, also defined as conditioning. Thereby, microbial conditioning indirectly promotes leaf litter decomposition though the stimulation of shredders' feeding activity (Cummins, 1974; Bärlocher & Kendrick, 1975), which ultimately results in the production of fine particulate organic matter that is an essential resource for collectors and deposit-feeding organisms (Bundschuh & McKie, 2016). Driven by this crucial role in stream food webs, changes in leaf-associated microbial communities can have far-reaching ecological consequences (M. O. Gessner et al., 2010).

The structure of leaf-associated microbial communities is shaped by their surrounding environment, including chemicals of anthropogenic origin (Canhoto, Gonçalves & Bärlocher, 2016). A repeated or continuous exposure to anthropogenic chemicals favours the occurrence of tolerant species with consequences for the communities' functioning (Blanck, 2002; Feckler et al., 2018). Indeed, laboratory studies suggest that constant exposure to antimicrobial substances, such as fungicides, can affect leaf palatability (Fernández et al., 2015; Zubrod et al., 2015) and leaf nutritional quality for shredders (Wallace et al., 2015; Zubrod et al., 2015b; Konschak et al., 2020). It remains, however, unclear whether agricultural field relevant exposure patterns, amongst others characterized by repeated fungicide exposures (Zubrod et al., 2019), can modify both the leaf-associated microbial community and the nutritional quality of leaves for shredders.

At the same time, the leaf species identity may function as an additional filter for microbial communities due to their unique recalcitrance and nutrient levels (e.g., Cornwell et al., 2008; Hladyz et al., 2009; Swan, Gluth & Horne, 2009; Frainer et al., 2016; Grossman, Cavender-Bares & Hobbie, 2020; Wang et al., 2020). In fact, most studies assessing impacts of chemicals on leaf-associated microbial communities have been performed with black alder (*Alnus glutinosa* (L.) GAERTN.) leaves, which are characterised by high nitrogen and phosphorous concentrations (Gulis, 2001) combined with a low degree of recalcitrance (Melillo, Aber & Muratore, 1982; Malanson, 1993; Gulis, 2001). Consequently, this leaf species likely supports microbial growth

and activity through a relatively easy access to nutrients (Gulis, 2001). It may therefore be questioned whether effects of chemicals observed using black alder are transferable to leaf species of a lower quality, characterised by low nutrient concentrations or a high degree of recalcitrance.

To address this knowledge gap, we assessed bottom-up effects on shredders by focusing on leaf-associated microbial communities from distinct streams, one pristine site (P) and one site characterised by repeated fungicide exposure in viticulture (V; Fernández et al., 2015), conditioning two leaf species and their mixture. As leaf species we selected black alder and European beech (*Fagus sylvatica* L.), representing a low and high degree of recalcitrance, respectively (Gulis, 2001; Artigas et al., 2012). Leaf-associated microbial communities were characterised by their exoenzyme activity as a functional endpoint, and AH species composition as well as fungal and bacterial biomasses using species- and group-specific quantitative real-time polymerase chain reaction (qPCR) assays, respectively. Subsequently, those conditioned leaves were offered as food to *Gammarus fossarum* (KOCH) over 21 days. Responses of male and female *Gammarus* were assessed by measuring their growth rate in terms of biomass increase, feeding rate and faeces production, as well as their energy reserves in the form of neutral lipid fatty acid (NLFA) profiles (was only assessed for male individuals). The use of both sexes is motivated by the deviating life history strategies and thus ecological roles in ecosystems (e.g., Pöckl & Humpesch, 1990). Nonetheless, a transferability of results between sexes has been assumed (Naylor at al.,1989; Malbouisson et al., 1995). We hypothesised that i) independent of the exposure history of the microbial community, low quality leaf species (i.e., beech) will be mostly conditioned by AH species that are conjectured as capable of degrading highly recalcitrant material (Baudy, Zubrod, Konschak, Kolbenschlag, et al., 2021). Since published evidence (e.g., Feckler et al., 2018; Bundschuh et al., 2011) suggests that those species are more tolerant to fungicides (due to the land use around their sampling site), the hypothesised pattern of microbial colonization should be especially pronounced for the pre-disturbed (V) community when compared to the pristine (P) community. At the same time, these more tolerant fungal species that are able to degrade highly recalcitrant material (e.g., Baudy et al., 2021), represent a less nutritional food for shedders (Arsuffi & Arsuffi & Suberkropp, 1989; Graça et al., 2001), which will be reflected in a lower food intake, growth rate and altered NLFA profile in both *Gammarus*' sexes. On the other hand, ii) the higher nitrogen concentration and lower recalcitrance of alder leaves will enable AH species with a more limited ligninolytic enzymatic capability to colonise such leaves, compensating for potential differences in palatability of microbial communities from the P- relative to V-community. Consequently, alder leaves should provide a comparatively high-quality food for *Gammarus* through higher fungal biomass and diversity. Moreover, iii) the mixture of leaf species increases AH diversity because of increasing habitat diversity (M. O.

Gessner et al., 2010). At the same time, the anticipated lower food quality of beech leaves is compensated by a stimulated feeding on alder leaves, which is reflected by a higher *Gammarus* growth rate. Finally, it was hypothesised that iv) the responses of male and female gammarids to the different food qualities are comparable.

2. MATERIAL AND METHODS

2.1 General study design

We used a 2x3x2-factorial design, where the first factor was the exposure history of the leaf-associated microbial communities sampled from streams dominated either by forest (mainly beech; pristine – P; P-community) or agricultural (vineyard run-off – V, without riparian vegetation; V-community) land use in their catchment, which is supported by earlier publications (Fernández et al., 2015; Schneeweiss et al., 2022). The second factor refers to the leaf species (i.e., alnus and beech) and their mixture, colonised by two leaf-associated microbial communities served as inoculum and the third to *Gammarus* sex. The leaf-associated microbial communities were characterised through group- or species-specific qPCR as well as their enzymatic activity. In addition, the conditioned leaf material served as food for *Gammarus* (males and females) in a 21-day lasting feeding assay (n=40; Fig. 1). The impact on *Gammarus*' growth rate, absolute feeding rate, faeces production and NLFA profile were assessed.

2.2 Sources and procedures of leaf material and microbial communities

The study was initiated in March 2021 largely following published protocols (Zubrod, Bundschuh & Schulz, 2010) . Briefly, stream water was collected from: a pristine stream (P; Hainbach, Germany, 49° 14' N, 8° 09' E) dominated by forest originated in the nature conservation area (Palatinate Forest Nature Park); and a stream in the agricultural landscape – namely viticulture – with a known history of fungicide exposure as documented elsewhere (V; Modenbach, Germany, 49°25'N, 8°11'E; see more detailed information on chemical characterization in supplementary information, SI, A.1 Table S1-S5; Fernández et al., 2015; Schneeweiss et al., 2022; Landesamt für Umwelt, 2016). The temperature of stream water at the time of sampling was between 8.0 and 8.8ºC. The leaves were collected at the time of leaf fall in autumn 2019 close to Landau, Germany (49° 11' N 8° 7' E) and stored at -20°C until use. The conditioning was realised in separate 50-L stainless-steel channels, kept at 20 ± 1 ºC in darkness under permanent aeration inducing water movement, for 14 days with a water exchange, freshly collected from the stream, after seven days. Each channel contained, 25 L stream water used to colonise 500 g of unconditioned alder or beech leaves as well as their mixture (250 g of each leaf species). This procedure resulted in six food sources (two inocula

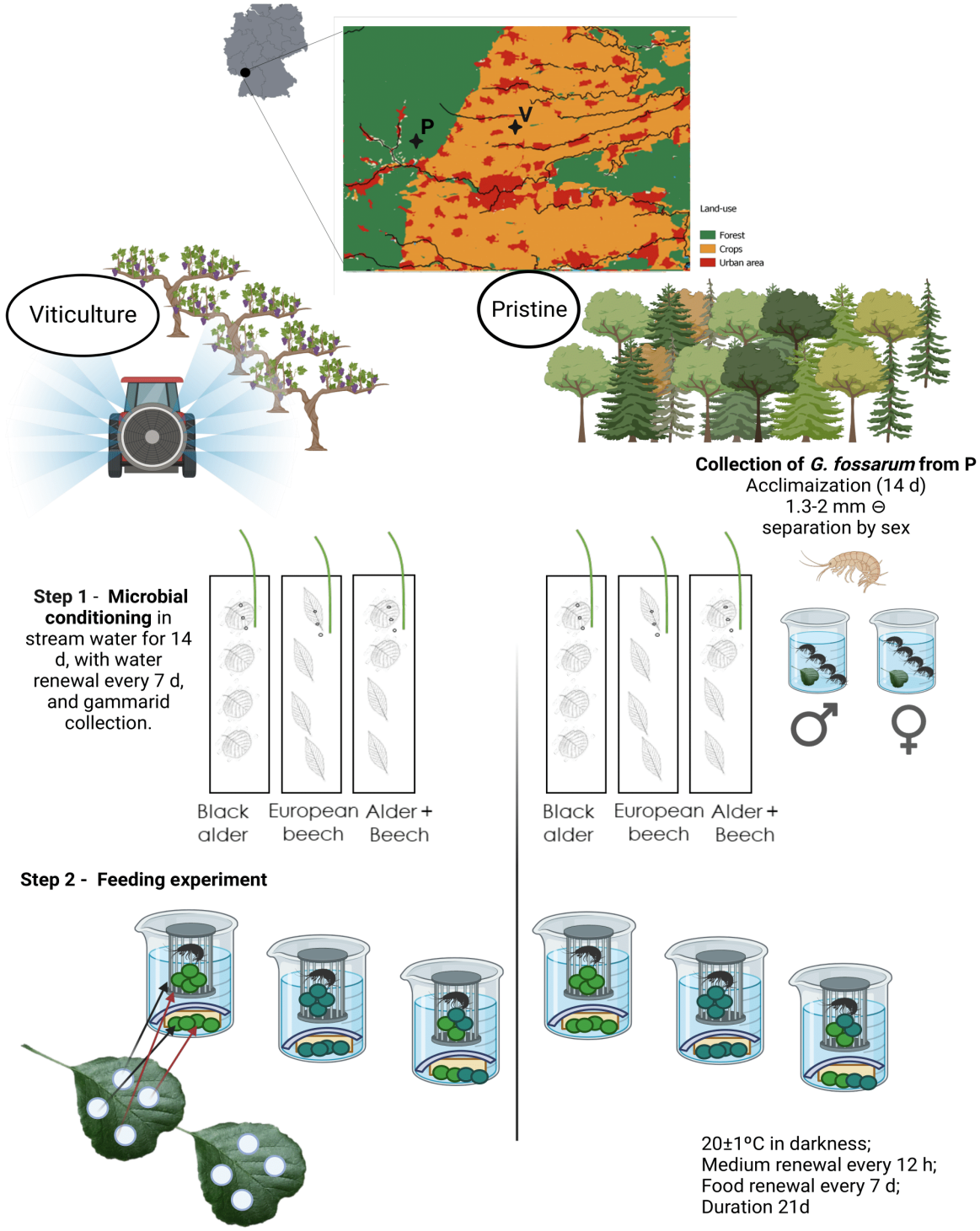


Figure 1 – Schematic overview of the study design. Step 1: Preparation for the feeding experiment: generating inocula and collecting test organisms – sampling stream water and *Gammarus fossarum* from a near-natural stream (pristine, P- community). Simultaneously, a stream surrounded by viticulture (V- community) was sampled. In the laboratory, the stream water was used to microbially colonize alder and beech leaves or a mixture of both in stainless steel channels under continuous aeration (green lines). Gammarids were separated by diameter and sex and kept in aerated medium, while fed with alder leaves *ad libitum* during acclimatization (14 d). Step 2: 21 d feeding experiment with a 2x3-factorial design (n=40). Per replicate 8 discs (Ø=16 mm) were cut of leaves generated in step 1, here only exemplified for alder treatment. Four leaf discs of each leaf species combination were fed to each gammarid, and another 4 leaf discs were used to control for leaf mass loss (orange rectangle), separated by a watch glass (grey line).

crossed with two leaf species and their mixture) provided to the test species *G. fossarum* (20 males and 20 females) as food source over 21 days (Fig.1). The conditioning was repeated weekly, including stream water collection (i.e.,7d and 14d after the initial colonization), ensuring the provisioning of food with comparable quality over the entire study duration.

2.3 Long-term feeding assay

Coinciding with the first stream water sampling, *G. fossarum* were collected from the Hainbach. In the laboratory, *Gammarus* were passively size separated using sieves with decreasing mesh sizes (Franke, 1997). Adults passing a sieve with a mesh size of 2.0 mm but being retained by 1.3 mm were selected for this experiment. Specimen were subsequently separated by sex, identified by their position in pre-copula pairs (Fielding et al., 2003; Pascoe et al., 1995). *Gammarus* were kept in aerated test medium (SAM-5S; Borgmann, 1996) for 14d and acclimatized to 20 ± 1 ºC in darkness while being fed *ad libitum* with unconditioned black alder leaves, ensuring *Gammarus* had access to a good quality food source (Bloor, 2011).

During the feeding assay, *Gammarus* were offered six food sources as detailed in section 2.2. Therefore, eight leaf discs (Ø =16 mm) were cut from two conditioned leaves, to ensure comparable results on the leaf mixture treatment, including one leaf from each species, and allocated to one replicate, with 40 replicates (20 male plus 20 female gammarids) being prepared for each treatment (Fig. 1). Each replicate consisted of a 250-mL glass beaker and was equipped with a cylindrical mesh cage made from stainless-steel (mesh size: 0.5 mm) containing one *Gammarus* and four leaf discs (two from each leaf). A second, rectangular mesh cage contained the remaining four leaf discs controlling for microbial leaf mass loss. A watch glass separated these two cages preventing adhesion of *Gammarus'* faeces to the leaf discs in the rectangular cage (see Zubrod et al*.,* 2015b; Fig.1). Replicates were filled with 250 mL test medium (SAM-5S; Borgmann, 1996), which was automatically renewed twice a day. The flowrate was selected to not remobilise the faeces, which was identified during a preliminary experiment. Moreover, every seventh day, remaining leaf discs and faeces were retrieved and gammarids were translocated to a new beaker with fresh medium and fresh leaf discs. The remaining leaf discs from each cage were collected, dried at 60 °C for 24 h and weighed to the nearest 0.01 mg. The old medium was filtered through pre-weighed glass fibre filters (GF/6, Whatman, Dassel, Germany), dried and weighed as detailed above to determine faeces production. At the termination of the experiment (after 21 days), surviving *Gammarus* (mortality did not exceed 5%) were shock frozen in liquid nitrogen and stored at −80 °C before being freeze-dried and weighed to the nearest 0.01 mg. Those organisms were used to determine growth rates and assess the NLFA profile of five randomly chosen male *Gammarus* per treatment (section 2.5). The sole focus on male *Gammarus* is motivated by the endeavour to reduce intra-treatment variability (Pascoe et al*.,* 1995; Fielding et al*.,* 2003). Similarly, leaf

discs (after 7 days in the test system with *Gammarus*) from the rectangular cage of five randomly chosen replicates were frozen at -20 °C for further analysis. Two of these leaf discs were used to assess microbial community composition (section 2.4.1) and the remaining two leaf discs served the activity analyses of exoenzymes (section 2.4.2). Replicates containing dead *Gammarus* (not exceeding 5%) were excluded from any analyses.

2.4 Characterisation of the leaf-associated microbial communities

2.4.1 Quantitative real-time PCR

DNA was extracted using the FastDNA® Spin Kit for Soil in combination with the FastPrep™-24 5G Instrument (MP Biomedicals, Germany) generally according to the manufacturer's protocol. Fungal and bacterial DNA was quantified following Baudy et al. (2019) and Manerkar, Seena & Bärlocher (2008) using qPCR reactions. On the species level (10 common and co-occurring AH species; Zubrod et al., 2015), the amount of DNA was measured as a proxy for fungal biomass based on species-specific TaqMan® qPCR reactions (Applied Biosystems, USA). On the group level, the amount of fungal and bacterial operon copies was measured as a proxy for overall fungal and bacterial biomass via SYBR® Green reactions slightly adapted (Manerkar, Seena & Bärlocher, 2008). PCR reaction mixtures were prepared with 2.8 µL of distilled water, 0.1 µL of forward primer, 0.1 µL of reverse primer, 2 µL DNA extract, and 5 µL of master mix PowerUp™ SYBR® Green, (Applied biosystems). PCR reactions consisted of initial denaturation at 95 °C for 2 min, followed by denaturation at 95 °C for 15 s, annealing at 55 °C for 15 s, and extension at 72 °C for 60 s for 40 cycles. Both types of qPCR reactions were performed on a Mastercycler® ep gradient S (Eppendorf, Germany) using 0.2-mL 8-tube strips covered with clear optical 8-cap strips (Sarstedt AG & Co. KG, Nümbrecht, Germany). More details on the assays and data analyses are provided in the Supplementary Information (A2; Table S6 and S7).

2.4.2 Exoenzyme activity

To quantify hydrolases' and oxidases' activities, we use the method described by DeForest (2009) but modified for leaf litter (see Baudy et al., 2020); detailed information on enzyme names, respective substrates, and targets is provided in the Supplementary Information A.2. Enzymatic activity was expressed as µmol of degraded substrate/mg leaf dry weight/hour (DeForest, 2009). Further details on substrate concentrations, plate layout and calculations can be found in Baudy et al. (2020). Additionally, we used enzyme activities to calculate the recalcitrance ratio of the leaf material, after square root transformation to reduce the effect of dominant enzyme activities, as normalised oxidases per total hydrolases activity (Table 2). The higher the ratio oxidase/hydrolase activity, the greater is the relative investment for degradation of recalcitrant carbon (Romero-Olivares et al., 2017).

2.5 Characterisation of *Gammarus*' physiological fitness

2.5.1 Growth, feeding and egestion rate

The individuals' growth rate was determined by subtracting the average (±sd) dry weight of 20 male (4.89 ± 1.06 mg) plus 20 female (3.00 ± 1.07 mg) lyophilized gammarids collected at the start of the bioassay, from the *Gammarus*' dry weight (after lyophilization) at test termination considering their respective sex, divided by the duration of the experiment (µg biomass gain/d). Although our approach to estimate growth might carry severe uncertainty, alternative approaches, such as the quantification of wet weight before and after the experiment substantially increases stress (unpublished studies point to a substantially higher mortality). The latter will potentially carry severer consequences for the data and conclusions that can be drawn thereof. The individuals collected at the start of the experiment were also used for NLFA profile analysis (see below) to which changes in NLFA profiles of all treatment groups have been related. The consumption of leaf material was calculated using the weight difference between the discs offered as food to the *Gammarus* in the cylindrical cage and those placed in the rectangular cage, divided by the final weight of the respective gammarid and time of the assay (i.e., 21 d; mg consumed leaf material/ mg Gammarus/d; Zubrod et al., 2011). Faeces production was calculated by subtracting the initial filter dry weight from the final filter dry weight divided by the final weight of the respective gammarid and time between food renewals (mg faeces/mg *Gammarus*/d; Zubrod et al., 2011).

2.5.2 Fatty acid analyses

Five randomly chosen male gammarids from each treatment plus five male individuals collected at the start of the bioassay were lyophilized and weighed to the nearest 0.01 mg for TAG FAs (Triacylglyceride fatty acids i.e., NLFAs) profiling following Bligh & Dyer (1959) and Konschak et al. (2020). We deliberately chose to analyse NLFAs, rather than phospholipid FA, as they are an important energy storage in invertebrates (Azeez et al., 2014) and are more readily affected by changes in the organisms' diet (Iverson, 2012). *Gammarus* were homogenized in a chloroform:methanol:water mixture (1:2:0.8; v:v:v). Subsequently, a TAG with three deuterated 18:0 FAs (Tristearin-D105, Larodan, Solna, Sweden) was added as internal standard, followed by chloroform and water addition to reach a chloroform:methanol:water ratio of 2:2:1.8 (cf. Bligh and Dyer, 1959). The samples were stored overnight at 4 °C. TAGs were separated from glycolipids and phospholipids by solid phase extraction (Chromabond® easy polypropylene columns, Macherey-Nagel, Düren, Germany; conditioned with 4 mL chloroform) and elution with 4 mL chloroform. Afterwards, the solvent was evaporated at 40 °C under a constant stream of nitrogen in a dry heat incubator (VLM Metall- blockthermostate, VLM GmbH, Bielefeld, Germany). TAGs were subsequently solved in 100 µL of dichloromethane and NLFAs were transesterified to fatty acid methyl esters

(FAME) using trimethylsulfonium hydroxide (Sigma-Aldrich, St. Louis, US-MO). FAME were analysed via gas chromatography with flame-ionization detection (GC-FID; Trace GC Ultra, Thermo Fisher Scientific, Bremen, Germany) using a Restek FAMEWAX column (30 m x 0.25 mm, 0.25 µm film thickness) and helium (1.4 mL/min) as carrier gas. FAMEs in each sample were determined using the retention times of FAME standards (37-component FAME Mix, Supelco CRM47885) and FAs were quantitatively analysed via external standard calibration (i.e., µg NLFA/mL). NLFA concentrations were corrected using extraction blanks and the recovery rate of the internal standard. The corrected NLFA concentrations were extrapolated to the total sample volume and normalized to *Gammarus*' dry weights (i.e., mg NLFA/g dry sample mass). The results are presented as difference relative to the subsamples of *Gammarus* collected at the start of the experiment.

2.6 Statistics and figures

Visual inspection, Shapiro–Wilk tests and Levene's tests were used to test for normality of the residuals and homoscedasticity of univariate data. When presumptions for parametric testing were met, two-factor or three-factor analyses of variance (ANOVA) were applied depending on the assessed variable (see Table S8-S10). As the presumptions for parametric testing were violated for data on the number of bacterial operon copies, a two-factor Kruskal-Wallis test, followed by a Bonferroni correction, was used to assess the individual and combined effect of the microbial communities' history and leaf species. Please note that considering the criticism of null-hypothesis significance testing we base our interpretation on both statistical significance and effect sizes (i.e., the difference between treatments (Newman, 2009; Feckler et al., 2018)).

Multivariate data (AH species composition and NLFA profiles) were square root-transformed to reduce the effect of dominant AH species or FAs (Happel et al., 2017). Afterwards, permutational multivariate analyses of variance (PERMANOVA) on transformed data were performed to assess the individual and combined impact of the microbial communities' history and leaf species, applying Bray-Curtis dissimilarities as a distance measure between groups. The assumption of homogeneous within-group dispersion was tested using the "betadisper" function and was fulfilled for all groups. Furthermore, AH species composition was displayed for graphical interpretation via non-metric multidimensional scaling plots using Bray-Curtis dissimilarities (NMDS; Clarke, 1993). Statistics and figures were conducted with R version 4.2.1 for Windows (R Core Team, 2022) as well as the add-on packages "vegan", "ggplot2", "multcomp", "rstatix" and "ggh4x". The graphical abstract was created in Biorender.com. Note that the term "significant(ly)" refers to statistical significance ($p<.05$) throughout the study.

3. RESULTS

3.1 Leaf-associated microbial communities

The number of fungal operon copies was lower (up to 40%) on beech and the mixture of alder and beech compared to alder alone. Although statistically not significant, this impact was more pronounced for the V- relative to the P-community (Tables 1 and S8-S10). Bacterial operon copies were three-fold more abundant on leaves in the mixture conditioned by the P- compared to the V-community (Table 1), but the difference was not statistically significant (Table S8).

Table 1. Mean (with 95 % confidence intervals; 10^8/mg leaf dw; n=3, fungal and bacterial operon copies of microbial communities colonizing the leaves used as food for G. fossarum during the 21-d lasting feeding assay. P: pristine; V: vineyard run-off.

Organism group	Endpoint	Treatment					
		alder-P	alder-V	alder-beech-P	alder-beech-V	beech-P	beech-V
Fungi	Operon copies/mg leaf dw	4.66 ± 3.30	6.78 ± 6.73	5.33 ± 3.6	3.44 ± 3.47	3.76 ± 3.43	3.56 ± 3.2
Bacteria		0.51 ± 0.92	1.72 ± 2.03	1.67 ± 1.22	0.59 ± 0.59	0.71 ± 0.73	0.58 ± 0.56

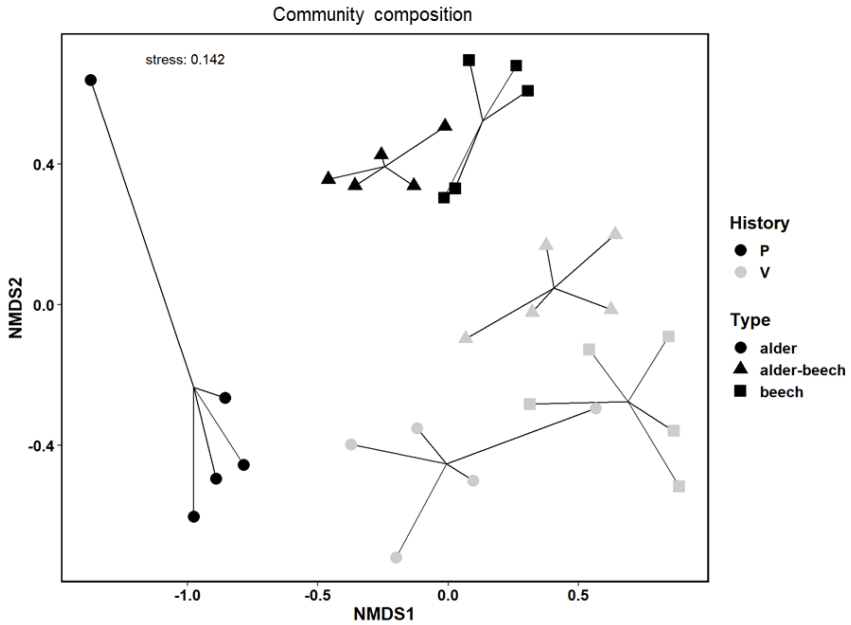


Figure 2 – Non-metric multidimensional scaling (NMDS) plot for leaf-associated aquatic hyphomycete communities. Leaf species are indicated by symbols (alder = circles, beech = squares, the mixture of both = triangles). Colours indicate the source of microbial inocula: pristine stream water (P) = black and vineyard run-off stream water (V) = grey. Spider webs connect the samples of each treatment at their respective group centroid. The stress value is provided as a measure of "goodness-of-fit" for NMDS, with a reasonable fit indicated when below 0.2 (Clarke,1993).

The AH community composition assessed through the quantification of DNA of 10 species, showed a difference between treatments. In fact, the factors community history (P vs V; p=0.004), leaf species (p=0.001) and an interaction between leaf species and community history (p=0.048; Fig. 2, Table S10; S12; S13) had a statistically significant impact in the community composition. Some species, such as *Alatospora acuminata* and *Flagellospora curvula*, were present in all treatments but with ~70% significantly lower abundance on beech leaves conditioned by the V- relative to the P-community was detected, these results suggest a shift in the relative contribution of individual species to the AH community (Tables S12-15).

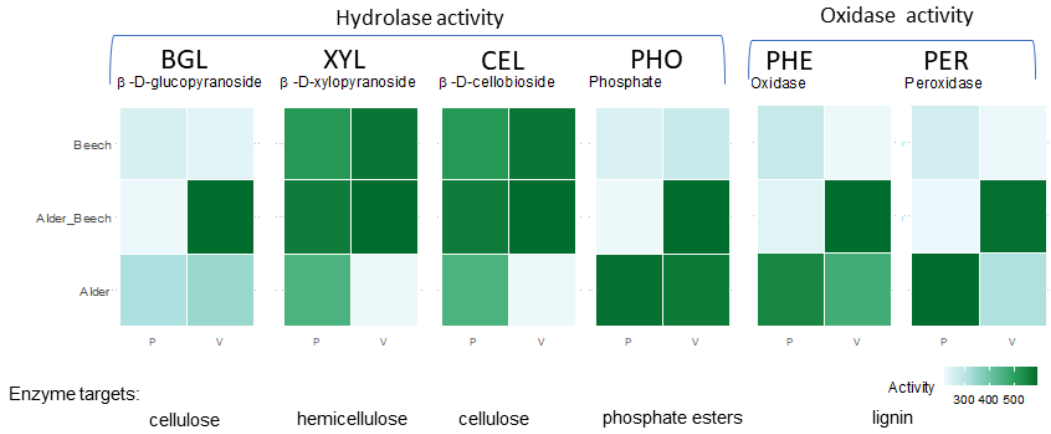


Figure 3 – Heatmaps displaying square root-transformed activities (µmol of degraded substrate/g leaf dry mass/hour) of β-1,4-glucosidase (BGL; targeting cellulose), β-1,4-xylosidase (XYL; targeting hemicellulose), cellobiohydrolase (CEL; targeting cellulose), phosphatase (PHO; targeting phosphate esters), phenol oxidase (PHE; targeting lignin) and peroxidaseperoxidase (PER; targeting lignin). Leaf species are shown on the Y-axis, while the community histories are shown on the x-axis (P: pristine; V: vineyard run-off).

A distinct pattern of the overall enzymes' activity was found for each of the treatments (Fig. 3) with only one enzyme (namely peroxidase) showing a significant interaction of microbial community history and leaf species (p=0.016; Table S9). Higher ligninolytic activity was found in all treatments conditioned by the V- compared to the P-community. Additionally, beech-associated microbes showed a higher hydrolase activity. On the contrary, alder-associated microbes showed a higher enzyme activity targeting phosphate esters and lignin (see also Table S16; SI A.3). The recalcitrance ratio (Table 2) of alder and beech leaves conditioned by the P-community was about 30% higher relative to their counterparts conditioned with the V-community. However, the opposite was observed in the mixture of alder and beech leaves, where the recalcitrance ratio of leaves conditioned by the P-community were 25% lower relative to the V-community. Moreover, alder leaves had overall the highest recalcitrance ratio.

Table 2. Investment in recalcitrant carbon degradation calculated as the ratio of oxidases divided by total hydrolases using square-root transformed data. The lower the ratio the higher the relative investment in recalcitrant carbon degradation (Romero-Olivares, Allison & Treseder, 2017). P: pristine; V: vineyard.

Treatment	*Total hydrolases*	*Oxidases*	*Ratio oxidases/hydrolases*
alder-P	191.74	51.79	0.27
alder-V	231.89	45.56	0.20
alder-beech-P	138.92	18.42	0.13
alder-beech-V	310.54	54.86	0.18
beech-P	177.67	25.47	0.14
beech-V	134.07	13.44	0.10

3.2 *Gammarus'* physiological fitness

Gammarus' growth rate was significantly impacted by the leaf species (p=0.001, Table S9) and showed a significant interaction of leaf species and the sex (p=0.005; Table S9). Male gammarids grew faster when fed with alder compared to male gammarids fed with the mixture of alder and beech (up to 60% depending on the inoculum) and beech leaves only (up to 115% depending on the inoculum; Fig. 4a). In contrast, the growth rate obtained for female gammarids was in extreme cases 21 times higher when fed with the mixture of alder and beech leaves compared to treatments in which only one of the leaf species was offered – a pattern independent of the inoculum (Fig. 4d). Additionally, a negative average growth rates obtained for one of the treatments, with the magnitude of the effect in combination with the variation within the data set pointing towards a growth stagnation or a slight loss in weight (Fig.4a & b). This observation may also be a consequence of a methodological artefact of the method chosen to calculate growth (see section 2.5.1).

Moreover, the feeding rate of females was slightly (5-30%) but consistently and significantly higher than that of males (p=0.048; Table S9). *Gammarus'* feeding rate was significantly influenced by the leaf species (p=0.014) and the interaction of community history and leaf species (p=0.004; Table S9) suggesting a substrate-dependent role of the source of the microbial inoculum. Finally, the feeding rate showed a similar pattern among treatments for both sexes while the effect sizes were more pronounced for males (Fig. 4b).

While the feeding rate of female gammarids was higher than that of males, the reverse pattern was observed for the faeces production. Females produced with ~10-20% significantly less faeces than males (Fig. 4c, f; p=0.008; Table S9). Moreover, faeces production was – independent of sex and source of the microbial inoculum – higher when feeding on the mixture

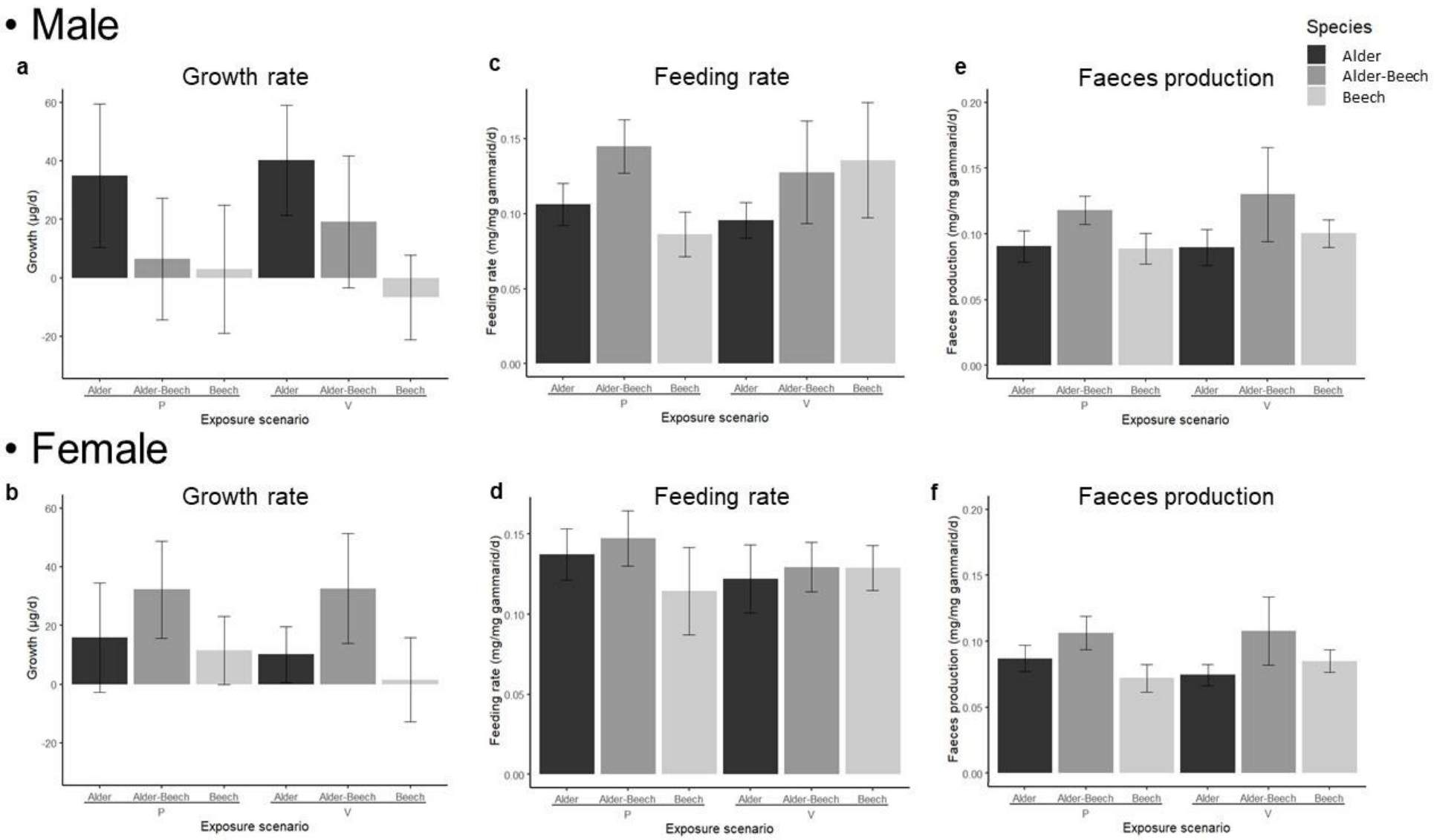


Figure 4. Mean (± 95% confidence intervals, n=20) a), b) growth rate as µg biomass gain/day, c), d) feeding rate as mg leaf material/mg gammarid/day, e), f) faeces production as mg faeces/mg gammarid/day of male and female gammarids, respectively, consuming alder (black), beech (light grey) or their mixture (dark grey) colonized by microbes with distinct exposure histories: P pristine; V vineyard.

of both leaf species (Fig. 4c, f). This observation is supported by a significant effect of the factor leaf species (p=0.0001, Table S9) and may be a consequence of a promoted feeding rate partially observed in those treatments (Fig. 4 b, e).

As displayed in Table 3, no significant differences among treatments in the NLFA profiles of male gammarids were found (Table S10). This includes all NLFA groups (saturated FAs, SAFA; monounsaturated FAs, MUFA; polyunsaturated FAs, PUFA) and biologically important FAs and their precursors, such as eicosapentaenoic acid (EPA; C20:5n-3), alpha-linolenic acid (ALA; C18:3n-3), and linoleic acid (LIN; C18:2n-6). Although the overall changes in NLFA profiles among treatments are statistically non-significant, gammarids have partly up to fifty percent lower levels of essential FAs and their precursors compared to the experiment initiation (see Table 3 for further details). While these changes suggest implications in the physiology of the organisms, the reliability of the observed trends needs further support by follow-up experiments.

Table 3. Percentage variation to the pre-experimental status of total, saturated (SAFA), monosaturated (MUFA) and polysaturated (PUFA) fatty acid content as well as linoleic acid (LIN; C18:2n-6), alpha-linolenic acid (ALA; C18:3n-3), and eicosapentaenoic acid (EPA; C20:5n-3) , that represent FA with biological interest (expressed as %total FA content per mg dry weight) of male *G. fossarum* subjected to different treatments during the 21-d lasting feeding assay. Statistical analyses are displayed in Table 1. P pristine; V vineyard run-off.

% Variation to pre-expriment (%FA/mg gammarid dw)	*NFLA*	*Treatment*					
		alder- P	alder-V	alder-beech-P	alder-beech-V	beech-P	beech-V
	TOTAL	-26.97	-11.62	0.12	-14.51	-27.73	-36.74
	SAFA	-23.61	-23.38	-12.39	-25.16	-24.94	-33.21
	MUFA	-20.58	0.29	9.59	2.42	-14.42	-32.67
	PUFA	-33.88	-18.03	-10.52	-28.82	-32.11	-20.27
	C18:2	-29.57	-17.20	4.32	-13.32	-30.99	-37.61
	C18:3	-50.80	-30.36	-24.11	-49.46	-38.64	9.18
	C20:5	-43.72	-35.58	-4.37	-35.68	-39.35	-19.96

4. DISCUSSION

Gammarus' physiology was partially affected by the tested combinations of leaf species and leaf-associated microbial communities with differing exposure histories. Beech leaves alone resulted, for both sexes and independent of the microbial community, in lower growth rates compared to alder leaves, with effect sizes being more pronounced for the V- than for the P-community, which supports our first hypothesis. In support of our second

hypothesis, alder (directly or indirectly) supports *Gammarus'* physiology more efficiently. Moreover, alder seems capable of compensating for the reduced presence of nutritional AH species in the beech-associated microbial community when offered together with beech (see hypothesis (iv)). Additionally, sex played a central role in the responses of *Gammarus* to the different treatments, which contradicts hypothesizes (iv). Consequently, extrapolation of responses among sex is not advisable. However, the partially high variability rendered some of the high effect sizes as statistically insignificant despite its potential biological relevance. Consequently, our strategy to base data interpretation on both statistical significance and effect sizes is further supported (Newman, 2008). Nonetheless, this strategy could introduce some uncertainty to our interpretation and discussion, which requires follow-up initiatives more specifically testing hypotheses that emerge based on the present study.

4.1 Leaf-associated microbial communities

The overall fungal and bacterial biomass, approximated by operon copies, were statistically insignificant among treatments suggesting a limited capacity of these parameters to explain the responses of gammarids' feeding. Although fungi and bacteria's chemical signals are considered attractive to shredders (Lange et al., 2005), the role of bacteria in their nutrition remains largely ignored. In contrast, literature suggests a preference of shredders for certain AH species (Arsuffi & Suberkropp, 1984). Indeed, in the present study the AH community composition varied significantly between P- and V-communities and among leaf species. The leaf associated microbial community, in particular AH community, is driving the palatability of leaf litter for shedders. However, no relation between shedders' preference and fungal biomass or enzymatic production could be established (Suberkropp et al., 1983). Instead, shedders' preferences for specific fungal species seems to be a function of the individual AH species traits, such as secondary metabolites (Arsuffi & Suberkropp, 1984), or mycelia's glyceride or FA content (Cargill et al., 1985; Arce Funck et al., 2015). Against this background, species considered more palatable (e.g., *A. acuminata, F. curvula*; (Arsuffi & Suberkropp, 1989; Suberkropp et al., 1983)) had equally high or higher biomasses on leaves conditioned by the P- relative to the V-community, independent on the leaf species. Those AH species are also assumed more nutritional (Arce Funck et al., 2015; Rong et al., 1995) to leaf-shredding organisms such as *Gammarus*. On the other hand, less nutritional AH species (such as *Tetracladium marchalianum or Tricladium angulatum*) were either absent or had a lower biomass on leaves conditioned by the P-community compared to leaves conditioned by the V-community. This pattern is in accordance with several studies (e.g., Bärlocher, 1973; Arsuffi & Suberkropp, 1989; Gonçalves et al., 2014), suggesting that more tolerant species, such as *T. marchalianum* (Maltby et al., 1995), ultimately dominate stressed AH communities (Bundschuh, Zubrod, Kosol, et al., 2011; Solé et al., 2008). Furthermore, AH species patterns

are less consistent among leaf species. *Neonectria lugdunensis* is either clearly dominating on alder conditioned by the P-community or is the second most abundant species when the V-community served as inoculum. This pattern is not confirmed for beech or the mixture of beech and alder. At the same time, *N. lugdunensis* is among the least preferred AH species for detritivores according to Arsuffi & Suberkropp (1989). Consequently, a generalizable pattern of AH community composition among substrates or the origin of the microbial inoculum is not abstractable, particularly as shedders' feeding preference for AH species is variable (e.g., Gonçalves et al., 2014). Moreover, we would like to highlight that laboratory conditions, which may include temperature differences relative to the field (Carl et al., 2022) and the presence of shredders' faeces (Díaz Villanueva et al., 2011), can impact microbial communities. By monitoring the succession of these communities over the study's duration, the magnitude of the effects could be quantified in future studies, further supporting a reasonable interpretation of the results presented here.

4.2 Responses of *Gammarus* to different food qualities

The fact that different leaf species presented different palatability should have had, according to our hypotheses, an impact on *Gammarus'* physiology. Based on *Gammarus'* growth, both sexes did not perform well when fed with beech only, a potential consequence of its higher recalcitrance and conditioning with less nutritional AH species, such as *N. lugdunensis*. Moreover, males and females showed different general growth patterns: despite the partially high variability within treatments, it may be abstracted that males and females grew faster when feeding on alder and the mixture of both leaf species, respectively, a pattern independent of the leaf-associated microbial community.

This observation of differing preferences may be explained by sex-specific requirements and life history strategies: although literature on this topic is scarce, studies have reported that male *Gammarus* live longer and have larger sizes than females with the aim to increase their competitiveness and support mate-guarding (Pöckl & Humpesch, 1990; Pöckl, 1992; Pöckl, Webb & Sutcliffe, 2003), suggesting that males strive for resources optimising their growth. Indeed, males grew faster when their feeding rate was the lowest (i.e., fed with alder) pointing to an efficient use of high-quality leaf litter additionally characterised by an AH community of presumably high nutritional quality. The introduction of beech into the leaf mixture decreases the food quality, as does the presumed nutritional quality of the AH community, leading to a higher feeding rate but lower growth of males. The latter indicates compensatory feeding, a mechanism by which organisms consume higher amounts of low-quality food to meet their nutritional requirements (Feckler et al., 2015; Rasmussen, Wiberg-Larsen, Baattrup-Pedersen, Friberg, et al., 2012). Although FA profiles did not show significant changes in male gammarids exclusively feeding on beech, highly unsaturated (essential) FAs, such as ALA and EPA, were

more strongly reduced compared to the test initiation. This observation was not confirmed when the mixture of both leaf species served as food. Even though data on female gammarids is lacking this observation supports the assumption that alder may compensate for lower food quality of beech leaves.

The generally lower NLFAs' concentration compared to individuals from the start of the bioassay, points towards the fact that gammarids were fed with lower quality food in the lab compared to the situation in the field, where they are able to supplement their dietary needs with other sources (e.g., algae; Guo et al., 2016; 2018) . Earlier studies have shown that laboratory conditions (e.g., changes in temperature, flux, or nutrient availability as for example derived from the amphipod faeces) can change the microbial community compared to field conditions (Carl et al., 2022). These changes in physical and chemical conditions potentially select more tolerant species, with potential implications in food quality as explained in the previous section. These more tolerant fungal species are often less palatable to *Gammarus*, potentially interfering with their feeding and physiology. This calls for further efforts to quantify the impact of such confounding factors, for example through the monitoring of the succession within the microbial community over the study duration. Moreover, the experiment was initiated in March and thus prior to the usual first fungicide application of the growing season. This fact points to the possibility for recolonization of AH from less or even uncontaminated upstream sections influencing the V-community of our study as documented for invertebrates (Orlinskiy et al., 2015). At test initiation we assumed, however, a change in AH communities when sampled from streams in vineyards (i.e., V-community) due to repeated fungicide exposure over the last years or even decades. Consequently, and contrary to our assumption,-the impact of fungicide exposure in AH communities may be assumed to be buffered by recolonization over the winter season. Re-running the experiment during or shortly after the main fungicide application period may be recommended to capture a field relevant worst-case scenario.

In contrast to males, females increase their size to enhance fecundity and carry eggs (Pöckl, 1990, 1992), with the latter also affecting their mobility and thus ability to exploit food resources (Lewis & Loch-Mally, 2010). We, consequently, assume females will constantly feed on any leaf species available to survive and wait for better conditions supporting growth, moulting and brood development. Bakkar et al. (2017) supports our assumptions, demonstrating that male and female sesarmid crabs produced faeces with a different chemical signature when feeding on mangrove leaves, suggesting a sex-specific digestive process. Moreover, due to competitive behaviour (e.g., cannibalism as food preference over sex, Ward, 1983; Dick, Irvine & Elwood, 1990; Ward & Porter, 1993; Dick, 1995; Ironside et al., 2019) and size advantage of males over females, the latter may have evolved to use a mixed quality of food, which is reflected by the efficient use of recalcitrant leaves in the present study. While this

assumption needs further verification also in the field, it points to the fact that an extrapolation – also at the physiological level – from males to females (commonly used in previous studies due to reduced intra-treatment variability; Pascoe et al., 1995; Fielding et al., 2003) is not straightforward and needs particular attention because of their relevance for population development.

Overall, the present study suggests that the leaf species identity, and thus the substrate on which the microbial communities grow, has a larger impact on the physiology of the next trophic level (i.e., the shredders) than the microbial community as such. As this observation is based on a fairly limited number of community history replicates (i.e., one P-community and one V-community), its general applicability needs further scrutiny.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

The interaction of leaf species and community history shaped the leaf-associated AH community composition. This stirs up a sex-specific change of gammarids' fitness as shown by differences in their growth. Particularly the sex-specific response to the different substrates questions the procedure of earlier studies using either only one sex or not being able to differentiate sex. Consequently, sex-specific responses are not yet properly considered. Moreover, the lack of a clear pattern in energy reserves on males (here the NLFA profile) calls not only for expanding replication but also the use of both sexes in physiological assessment, which is supported by the sex-specific growth pattern in response to the food sources. Thereby, a more comprehensive pattern on potential bottom-up related effects in the wider food web can be developed.

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Data Availability Statement: Data are available from the authors upon reasonable request.

Conflict of interest statement: The authors do not see a conflict of interest with the publication of this paper.

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Supplementary information for

Microbial community history and leaf species shape bottom-up effects in a freshwater shredding amphipod

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A.1 Physical and chemical parameters of the studied region

Table S1 - Information on environmental parameters of the pristine stream (P; Hainbach, 49° 14' N, 8° 09' E) and a stream in the agricultural landscape – (V; Modenbach, 49°25'N, 8°11'E), adapted from Gonçalves et al. (submitted). Nutrient concentrations were analysed on-site with Visocolor® test kits (Macherey-Nagel, Düren, Germany). Water temperature, pH, electrical conductivity and dissolved oxygen were measured using a multiparameter analyser Multi 340i (WTW, Weilheim, Germany) and flow velocity was measured with a flow meter (Höntzsch, Waiblingen, Germany).

Site	Parameters	Sampling date		
		11.04.19	18.04.19	25.04.19
P	**pH**	7.64	7.37	7.89
Hainbach	**Temperature (Cº)**	8.9	8.2	11.7
	Conductivity (µS/cm)	123	119	123
	O_2 (%)	96.47	111	128
	O_2 (mg/L)	10.79		11.87
	NO_3 (mg/L)	0.2-0-7	0.2-0-7	0-0.2
	PO_4 (mg/L)	<0.15	<0.15	0.46
	P_2O_5 (mg/L)	<0.11	<0.11	0.34
		30.05.19	06.06.19	13.06.19
V	**pH**	8.06	8.03	8.28
Modenbach	**Temperature (ºC)**	12.6	15.2	15.5
	Conductivity (µS/cm)	400	363	397
	O_2 (%)	134	109.3	129.1
	O_2 (mg/L)		10.8	11.88
	NO_3 (mg/L)	20	10-20	20
	PO_4 (mg/L)	0.05	0.05	0.05

Table S2 – Characterization of the sampling region, which included 17 sampling sites in different streams covering a gradient of fungicide exposure, forest to vineyards (maximum distance of 4 km) during the summer of 2012, adapted from Fernández et al., 2015. Nutrient concentrations were analysed on-site with Visocolor® test kits (Macherey-Nagel, Düren, Germany). Water temperature, pH, electrical conductivity and dissolved oxygen were measured using a multiparameter analyser Multi 340i (WTW, Weilheim, Germany) and flow velocity was measured with a flow meter (Höntzsch, Waiblingen, Germany).

Variable	Minimum	Maximum	Median	Mean	SD
Stream width (m)	0.8	7.3	1.67	2.21	1.61
Stream depth (m)	0.07	0.43	0.15	0.19	0.1
Current velocity (m/s)	0.01	0.67	0.23	0.26	0.17
Temperature (°C)	11.21	13.77	12.62	12.5	0.81
pH	7.51	8.26	7.87	7.85	0.24
Oxygen (mg/L)	5.3	10.61	9.6	9.1	1.3
Conductivity (µS/cm)	110	1290	332	481	340
Nitrite (mg/L)	0	0.8	0.04	0.09	0.19
Nitrate (mg/L)	2	60	5	9	14
Phosphate (mg/L)	0.1	0.6	0.2	0.25	0.13
Ammonium (mg/L)	0	0.2	0	0.01	0.05

Table S3 - Frequency of detection of pesticides measured during summer 2012 by Fernández et al., 2015 in vineyard sites corresponding to the same sampling region as the present study.

Pesticide	Type	Detections (%)
Azoxystrobin	Fungicide	62
Boscalid	Fungicide	77
Cyprodinil	Fungicide	31
Dimethoate	Insecticide	23
Dimethomorph	Fungicide	77
Fenhexamid	Fungicide	69
Fludioxonil	Fungicide	46
Imidacloprid	Insecticide	23
Indoxacarb	Insecticide	53
Iprovalicarb	Fungicide	69
Kresoxim-methyl	Fungicide	62
Metalaxyl-M	Fungicide	85
Metrafenone	Fungicide	69
Myclobutanil	Fungicide	100
Pyrimethanil	Fungicide	70
Quinoxyfen	Fungicide	38
Tebuconazole	Fungicide	76
Tebufenpyrad	Fungicide	0
Tolyfluanid	Insecticide	0

Table S4 – Information on environmental variables characterising sites of Hainbach and Modenbach during summer 2019, adapted from Schneeweiss et al., 2022. Nutrient concentrations indicate the amount of nitrogen or phosphor in the respective compound (i.e. NH_4-N, NO_3-N, NO_2-N, PO_4-P).

Stream	Hainbach	Modenbach
Site type	Refuge	Agriculture
Stream width (m)	1.3	2.4
Stream depth (cm)	18	16
Flow velocity (m/s)	0.17	0.25
Water temperature (°C)	13.6	15.6
Dissolved oxygen (%)	91.1	91.9
Dissolved oxygen (mg/L)	9.03	8.97
Conductivity (µS/cm)	124	388
pH	7.51	7.45
NH_4-N (mg/L)	0.04	0.07
NO_3-N (mg/L)	0.87	2.98
NO_2-N (mg/L)	0.04	0.04
PO_4-P (mg/L)	0.03	0.06

Table S5 – Pesticides number and concentration in ng/L found on the interest sites during summer 2019, adapted from Schneeweiss et al., 2022.

Stream	Hainbach	Modenbach
Site type	refuge	agriculture
Number of detected pesticides	6	15
Total concentration [ng/L]	1.57	53.47

A.2 Methods & data analysis

Table S6 - Information on qPCR assay developed by Baudy et al. (2019): designations, targeted species, including the used model strain (DSM number from the German Collection of microorganisms and cell culture at the Leibniz institute-DSMZ) and template sequences' GenBank accession number as well as technical properties including length, melting temperature, guanine-cytosine content, binding region, and amplicon length.

ID	Target species	DSM number	GenBank accession number	Length (bp)	Melting temperature (ºC)	G-C content (%)	Binding region	Amplicon length (bp)
ALAC	*Alatospora acuminata*	104360	MH930815	21	59	52	ITS2	82
				21	59	52	ITS2/LSU	
				14	68	50	ITS2	
ARTE	*Articulospora tetracladia*	104345	MH930816	18	59	31	5.8S	77
				18	59.5	31	ITS2	
				18	68	39	5.8S/ITS2	
CLAQ	*Clavariopsis aquatica*	104362	MH930817	20	59	45	ITS2	82
				23	59.2	48	ITS2	
				16	70	56	ITS2	
CLLO	*Clavatospora longibrachiata*	104365	MH930818	24	59.6	42	ITS2	89
				29	59.6	34	ITS2	
				20	69	30	ITS2	
FLCU	*Flagellospora curvula*	104334	MH930819	22	57.8	50	ITS2	108
				20	58.1	60	ITS2	
				18	70	56	ITS2	
HEST	*Heliscella stellata*	104386	MH930820	22	58.9	50	ITS2	79
				25	58.3	36	ITS2	
				23	70	30	5.8S/ITS2	
LETE	*Lemonniera terrestris*	104344	MH930821	22	59.1	50	ITS2	81
				18	58.6	61	ITS2	
				17	70	53	ITS2	

NELU	*Neonectria lugdunensis*	104361	MH930822	24	59	50	ITS2	90
				22	58	50	ITS2/LSU	
				14	69	57	ITS2	
TEMA	*Tetracladium marchalianum*	104373	MH930823	24	58	50	ITS2	64
				20	58	55	ITS2	
				18	69	56	ITS2	
TRAN	*Tricladium angulatum*	104374	MH930824	20	58.5	50	5.8S/ITS2	129
				24	59	46	ITS2	
				14	68	64	ITS2	

Table S7 - Information on qPCR assay developed by Manerkar et al. (2008): Targeted group, primers (Baker & Cowan, 2003; White et al., 1990) used including the template sequences as well as technical properties including melting temperature, amplified region and length (bp).

Target	Primer	Sequence	Melting temperature (ºC)	Amplified region	Amplicon length (bp)
Fungi	ITS3F	GCATCGATGAAGAACGCAGC	55.3	5.8S and ITS2	400
	ITS4R	TCCTCCGCTTATTGATATGC			
Bacteria	E8F	AGAGTTTGATCCTGGCTCAG	55	16S	525
	E533R	TIACCGIIICTICTGGCAC			

Table S8- Output for statistical analyses for fungal and bacterial DNA copy numbers. df, degrees of freedom; SS, sum of squares; MS, mean squares.

Enpoint	*Method*	*Source of variation*	*df*	*SS*	*MS*	*F-value*	*p-value*
DNA fungal copies	ANOVA	Community history	1	$8.70x10^{26}$	$8.70x10^{26}$	1.63	0.214
		Leaf species	2	$7.32x10^{26}$	$3.66x10^{26}$	0.69	0.514
		Community history x Leaf species	2	$7.32x10^{26}$	$3.66x10^{26}$	0.69	0.514
		Residuals	24	$1.28x10^{28}$	$5.34x10^{26}$		
DNA bacterial copies		----------	chi-squared	df	p-value		
	Kruskal Wallis		4.27	5.00	0.51		

Table S9 - Output for statistical analyses for Peroxidase, growth, feeding rate and faeces production. df, degrees of freedom; SS, sum of squares; MS, mean squares. p-values printed in bold indicate statistical significance.

Enpoint	*Method*	*Source of variation*	*df*	*SS*	*MS*	*F-value*	*p-value*
Peroxidase PER	ANOVA	Community history	1	$1.29x10^5$	$1.29 x10^5$	0.03	0.861
		Leaf species	2	$1.94 x10^7$	$9.68 x10^6$	2.34	0.118
		Community history x Leaf species	**2**	**$4.07 x10^7$**	**$2.04 x10^7$**	**4.92**	**0.016**
		Residuals	24	$9.94x10^7$	$4.14 x10^6$		
Growth	ANOVA	Community history	1	0.00	0.00	0.00	0.999
		Leaf species	**2**	**11.7**	**5.86**	**7.09**	**0.001**
		Sex	1	0.07	0.07	0.09	0.772
		Community history x Leaf species	2	1.70	0.850	1.03	0.359
		Community history x Sex	1	0.39	0.39	0.47	0.494
		Leaf species x Sex	**2**	**9.13**	**4.56**	**5.52**	**0.005**
		Community history x Leaf species x Sex	2	0.17	0.09	0.11	0.900
		Residuals	216	178	0.83		
Feeding rate	ANOVA	Community history	1	0.00	$2.0x10^{-6}$	0.00	0.977
		Leaf species	**2**	**0.02**	**0.01**	**4.37**	**0.014**
		Sex	**1**	**0.01**	**0.01**	**3.97**	**0.048**
		Community history x Leaf species	**2**	**0.03**	**0.02**	**5.80**	**0.004**
		Community history x Sex	1	$4.40x10^{-3}$	$4.43x10^{-3}$	1.68	0.197
		Leaf species x Sex	2	$6.80x10^{-3}$	$3.38x10^{-3}$	1.28	0.281
		Community history x Leaf species x Sex	2	$3.20x10^{-3}$	$1.63x10^{-3}$	0.61	0.542
		Residuals	216	0.57	$2.65x10^{-3}$		
Faeces production	ANOVA	Community history	1	0.67	0.67	1.06	0.304
		Leaf species	**2**	**18.2**	**9.11**	**14.43**	**0.000**
		Sex	**1**	**4.59**	**4.59**	**7.27**	**0.008**
		Community history x Leaf species	2	1.73	0.87	1.37	0.256
		Community history x Sex	1	0.28	0.28	0.44	0.510
		Leaf species x Sex	2	0.20	0.10	0.16	0.857
		Community history x Leaf species x Sex	2	0.22	0.11	0.18	0.839
		Residuals	216	136	0.631		
		Total	33	1.661	1.000		

Table S10 - Output for statistical analyses for multivariate data, AH composition and fatty acids profile. df, degrees of freedom; SS, sum of squares; MS, mean squares. p-values printed in bold indicate statistical significance.

Enpoint	*Method*	*Source of variation*	*df*	*SS*	*MS*	*F-value*	*p-value*
AH composition	PERMANOVA	**Community history**	**1**	**0.81**	**0.08**	**3.48**	**0.004**
		Leaf species	**2**	**2.78**	**0.28**	**5.92**	**0.001**
		Community history x Leaf species	**2**	**0.77**	**0.07**	**1.65**	**0.048**
		Residuals	24	5.63	0.56		
SAFA	PERMANOVA	Community history	1	0.021	0.014	0.46	0.653
		Leaf species	3	0.148	0.099	1.08	0.354
		Community history x Leaf species	2	0.089	0.059	0.98	0.429
		Residuals	27	1.233	0.826		
		Total	33	1.491	1.000		
MUFA	PERMANOVA	Community history	1	0.004	0.003	0.09	0.920
		Leaf species	3	0.175	0.120	1.23	0.272
		Community history x Leaf species	2	0.028	0.019	0.30	0.863
		Residuals	27	1.249	0.857		
		Total	33	1.457	1.000		
PUFA		Community history	1	0.024	0.0112	0.36	0.788
	PERMANOVA	Leaf species	3	0.276	0.126	1.36	0.240
		Community history x Leaf species	2	0.062	0.028	0.46	0.832
		Residuals	27	1.823	0.834		
		Total	33	2.186	1.000		
Total NFLA		Community history	1	0.011	0.006	0.20	0.917
	PERMANOVA	Leaf species	3	0.188	0.113	1.21	0.256
		Community history x Leaf species	2	0.068	0.041	0.66	0.642
		Residuals	27	1.394	0.839		
		Total	33	1.661	1.000		

Table S11 - Mean of measured endpoints: fungal and bacterial 10^8DNA copy numbers; lipid fatty acid profile, saturated FAs, SAFA; monounsaturated FAs, MUFA; polyunsaturated FAs, PUFA, growth rate, feeding rate, faeces production ± sd.

Endpoint	Unit		Treatment																				
			alder-P			alder-V			alder-beech-P			alder-beech-V			beech-P			beech-V					
			mean	±	sd	mean	±	sd	mean	±	sd	mean	±	sd	mean	±	sd	mean	±	sd			
Fungi	Number of DNA copies /mg leaf dw		4.66	±	2.88	6.78	±	5.95	5.33	±	3.18	3.44	±	3.08	3.76	±	3.04	3.56	±	2.88	PRE -EXP		
Bacteria			0.51	±	0.81	1.72	±	1.79	1.67	±	1.08	0.59	±	0.52	0.71	±	0.65	0.58	±	0.49	mean	±	sd
SAFA	%SAFA/mg gammarid dw		32.17	±	7.84	29.77	±	4.92	26.82	±	1.22	27.85	±	4.77	28.55	±	2.47	29.32	±	2.79	29.31	±	1.71
MUFA	%MUFA/mg gammarid dw		26.38	±	2.53	26.92	±	1.06	27.61	±	2.52	30.69	±	4.99	28.23	±	2.31	30.32	±	6.56	25.61	±	2.14
PUFA	%PUFA/mg gammarid dw		41.453	±	6.87	43.31	±	4.05	45.57	±	1.72	41.46	±	5.10	43.23	±	1.35	40.39	±	6.01	45.08	±	2.23
TOTAL NFLA	Total NFLA % / mg gammarid dw		39.55	±	12.18	47.86	±	13.51	54.22	±	24.32	46.30	±	20.23	39.14	±	13.86	34.26	±	11.98	53.02	±	18.65
Growth rate	µg/d	Female	15.91	±	42.43	10.12	±	21.65	32.24	±	37.85	32.57	±	42.76	11.52	±	26.29	1.50	±	32.60			
		Male	35.05	±	55.98	40.24	±	42.90	6.50	±	47.44	19.21	±	51.55	2.98	±	49.93	-6.62	±	32.91			
Feeding rate	mg/mg gammarid/d	Female	0.15	±	0.04	0.12	±	0.05	0.15	±	0.04	0.13	±	0.04	0.12	±	0.06	0.13	±	0.03			
		Male	0.11	±	0.03	0.10	±	0.03	0.15	±	0.040	0.13	±	0.08	0.09	±	0.03	0.14	±	0.09			
Faeces production		Female	0.10	±	0.03	0.09	±	0.03	0.12	±	0.025	0.130	±	0.08	0.09	±	0.03	0.10	±	0.02			
		Male	0.90	±	0.02	0.08	±	0.02	0.11	±	0.029	0.108	±	0.06	0.07	±	0.02	0.09	±	0.02			

Table S12 - Means of AH species composition DNA quantity (ng DNA per mg of leaf dry weight) measured via qPCR and respective AH individual species biomass estimation following Baudy et al. (2019) in mg AH culture dry weight per ng DNA measured

Species	Treatment											
	alder-P (ng DNA /mg leaf dw)	Species biomass estimation (mg AH culture dw/ ng DNA)	alder-V (ng DNA /mg leaf dw)	Species biomass estimation (mg AH culture dw/ ng DNA)	alder-beech -P (ng DNA /mg leaf dw)	Species biomass estimation (mg AH culture dw/ ng DNA)	alder-beech- V (ng DNA /mg leaf dw)	Species biomass estimation (mg AH culture dw/ ng DNA)	beech-P (ng DNA /mg leaf dw)	Species biomass estimation (mg AH culture dw/ ng DNA)	beech-V (ng DNA /mg leaf dw)	Species biomass estimation (mg AH culture dw/ ng DNA)
	mean		mean		mean		mean		mean		Mean	
Alatospora acuminata	$3.41x10^{04}$	$6.21x10^{-07}$	$3.47x10^{-04}$	$6.31x10^{-07}$	$3.79x10^{-02}$	$6.91x10^{-05}$	$1.13x10^{-02}$	$2.06x10^{-05}$	$1.36x10^{-01}$	$2.48x10^{-04}$	$4.01x10^{-02}$	$7.30x10^{-05}$
Tetracladium marchalianum	0	0	$3.21x10^{-04}$	$1.51x10^{-06}$	$2.18x10^{-05}$	$1.03x10^{-07}$	$5.43x10^{-03}$	$2.56x10^{-05}$	0	0	$2.62x10^{-02}$	$1.23x10^{-04}$
Neonectria lugdunensis	$5.40x10^{-03}$	$1.42x10^{-05}$	$1.30x10^{-03}$	$3.40x10^{-06}$	$1.04x10^{-02}$	$2.71x10^{-05}$	$1.66x10^{-03}$	$4.34x10^{-06}$	$1.21x10^{-03}$	$3.16x10^{-06}$	$8.28x10^{-04}$	$2.17x10^{-06}$
Tricladium angulatum	0	0	$1.17x10^{-03}$	$6.81x10^{-06}$	0	0	$5.83x10^{-03}$	$3.41x10^{-05}$	0	0	$1.90x10^{-02}$	$1.11x10^{-04}$
Articulospora tetracladia	0	0	0	0	$2.06x10^{-02}$	$1.83x10^{-04}$	0	0	$4.85x10^{-04}$	$4.31x10^{-06}$	0	0
Flagellospora curvula	$4.58x10^{-05}$	$9.51x10^{-08}$	$7.91x10^{-05}$	$1.64x10^{-07}$	$2.03x10^{-02}$	$4.21x10^{-05}$	$1.36x10^{-02}$	$2.83x10^{-05}$	$1.54x10^{-02}$	$3.19x10^{-05}$	$7.66x10^{-04}$	$1.59x10^{-06}$
Clavatospora longibrachiata	0	0	$4.14x10^{-04}$	$7.86x10^{-07}$	$6.89x10^{-03}$	$1.31x10^{-05}$	$1.79x10^{-04}$	$3.39x10^{-07}$	$1.61x10^{-03}$	$3.06x10^{-06}$	$1.28x10^{-03}$	$2.43x10^{-06}$
Lemonniera terrestris	0	0	$1.04x10^{-04}$	$9.10x10^{-07}$	$1.68x10^{-03}$	$1.46x10^{-05}$	$9.55x10^{-04}$	$8.32x10^{-06}$	$3.14x10^{-03}$	$2.74x10^{-05}$	$3.74x10^{-03}$	$3.26x10^{-05}$
Heliscella stellata	0	0	0	0	$2.55x10^{-02}$	$1.55x10^{-04}$	0	0	$1.62x10^{-03}$	$9.81x10^{-06}$	0	0

Table S13 – The contribution (in %) of each AH species to the community based on biomass estimated using qPCR (Table S4) separated by treatment. P: pristine; V: vineyard run-off.

AH species	% species contribution					
	alder - P	alder - V	alder – beech - P	alder – beech - V	beech - P	beech - V
Alatospora acuminata	4.17	4.44	13.70	16.92	75.72	21.09
Tetracladium marchalianum	0.00	10.65	0.02	21.04	0.00	35.64
Neonectria lugdunensis	95.19	23.91	5.38	3.57	0.96	0.63
Tricladium angulatum	0.00	47.91	0.00	28.03	0.00	32.06
Articulospora tetracladia	0.00	0.00	36.31	0.00	1.31	0.00
Flagellospora curvula	0.64	1.16	8.35	23.31	9.74	0.46
Clavatospora longibrachiata	0.00	5.53	2.59	0.28	0.93	0.70
Lemonniera terrestris	0.00	6.40	2.90	6.85	8.34	9.42
Heliscella stellata	0.00	0.00	30.74	0.00	2.99	0.00

Table S14 - Output for statistical analyses (Kruskal-Wallis and Pairwise Wilcox test with p-value adjustment BH) of the AH species biomass.

Species	Kruskal-Wallis		Pairwise Wilcox test					
				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
Tetracladium marchalianum	chi-squared	16.373	alder-beech-V	0.105				
	df	5	alder-P	0.441	**0.089**			
	p-value	**0.006**	alder -V	0.441	0.0252	0.0252		
			beech-P	0.441	**0.089**		0.0252	
			beech-V	0.105	0.317	**0.089**	0.314	**0.089**
Neonectria lugdunensis				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
	chi-squared	13.868	alder-beech-V	**0.060**				
	df	5	alder-P	0.377	0.422			
	p-value	**0.01647**	alder -V	**0.056**	1	0.422		
			beech-P	**0.056**	0.797	0.422	1	
			beech-V	**0.056**	0.563	0.272	0.422	0.422
Alatospora acuminata				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
	chi-squared	18.693	alder-beech-V	0.226				
	df	5	alder-P	**0.03**	**0.03**			
	p-value	**0.002**	alder -V	**0.03**	**0.03**	1		
			beech-P	**0.971**	0.526	0.151	0.126	
			beech-V	**0.422**	**0.068**	**0.03**	**0.03**	1
Heliscella stellata				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
	chi-squared	23.85	alder-beech-V	**0.029**				
	df	5	alder-P	**0.029**				
	p-value	**0.0002**	alder -V	**0.029**				
			beech-P	0.31	**0.017**	**0.017**	**0.017**	
			beech-V	**0.029**				**0.017**
Articulospora tetracladia				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
	chi-squared	25.433	alder-beech-V	**0.017**				
	df	5	alder-P	**0.017**				
	p-value	**0.0001**	alder -V	**0.017**				
			beech-P	**0.017**	0.424	0.424	0.424	
			beech-V	**0.017**				0.424
Flagellospora curvula				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P

	chi-squared	23.208	alder-beech-V	0.485				
	df	5	alder-P	**0.024**	**0.024**			
	p-value	**0.0003**	alder -V	**0.024**	**0.024**	0.841		
			beech-P	0.188	0.841	**0.024**	**0.024**	
			beech-V	**0.024**	**0.03**	0.075	0.091	0.083
Clavatospora longibrachiata				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
	chi-squared	9.5066	alder-beech-V	0.554				
	df	5	alder-P	0.095	0.216			
	p-value	0.09	alder -V	0.819	0.84	0.095		
			beech-P	0.917	0.544	0.095	0.819	
			beech-V	0.917	0.544	0.095	0.576	0.917
Lemonniera terrestris				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
	chi-squared	18.753	alder-beech-V	0.797				
	df	5	alder-P	0.095	0.056			
	p-value	**0.02**	alder -V	0.227	0.26	0.3		
			beech-P	0.797	0.631	0.056	0.103	
			beech-V	0.807	0.747	0.095	0.227	1
Tricladium angulatum				alder-beech-P	alder-beech-V	alder-P	alder -V	beech-P
	chi-squared	25.871	alder-beech-V	0.015				
	df	5	alder-P		0.015			
	p-value	**<0.0001**	alder -V	0.03	0.061	0.03		
			beech-P		0.015		0.03	
			beech-V	0.015	0.15	0.015	0.027	0.015

Table S15 - Output for simper analysis of community composition.

Treatment	**beech-P x alder-beech-P**	
Species	**Cumsum**	**p-value**
Articulospora tetracladia	0.561	**0.001**
Heliscella stellata	0.727	0.175
Alatospora acuminata	0.863	0.506
Lemonniera terrestris	0.94	0.856
Flagellospora curvula	0.97	0.834
Neonectria lugdunensis	0.994	0.837
Clavatospora longibrachiata	1	0.518
Tetracladium marchalianum	1	0.997
Tricladium angulatum	1	1
Treatment	**beech-P x alder-P**	
Alatospora acuminata	0.414	**0.001**
Lemonniera terrestris	0.615	**0.001**
Heliscella stellata	0.801	**0.053**
Neonectria lugdunensis	0.899	0.198
Flagellospora curvula	0.968	**0.032**
Articulospora tetracladia	0.985	0.892
Clavatospora longibrachiata	1	**0.008**
Tetracladium marchalianum	1	0.998
Tricladium angulatum	1	1
Treatment	**beech-P x beech-V**	
Tricladium angulatum	0.34	**0.045**
Tetracladium marchalianum	0.568	**0.024**
Alatospora acuminata	0.752	0.268
Lemonniera terrestris	0.896	0.185
Heliscella stellata	0.949	0.604
Flagellospora curvula	0.978	0.864
Articulospora tetracladia	0.99	0.934
Clavatospora longibrachiata	0.995	0.624
Neonectria lugdunensis	1	1
Treatment	**alder-beech-V x alder-V**	
Tricladium angulatum	0.415	**0.028**
Tetracladium marchalianum	0.589	0.257
Flagellospora curvula	0.716	**0.002**
Lemonniera terrestris	0.836	0.602
Alatospora acuminata	0.943	0.731
Neonectria lugdunensis	0.995	0.595
Clavatospora longibrachiata	1	0.0694
Heliscella stellata	1	0.99
Articulospora tetracladia	1	0.936

Treatment	alder-beech-V x alder-beech-P	
Articulospora tetracladia	0.579	**0.001**
Heliscella stellata	0.752	0.105
Tricladium angulatum	0.83	0.974
Lemonniera terrestris	0.873	0.981
Tetracladium marchalianum	0.915	0.882
Alatospora acuminata	0.946	0.996
Flagellospora curvula	0.972	0.842
Neonectria lugdunensis	0.995	0.815
Treatment	**alder-beech-V x beech-V**	
Tricladium angulatum	0.379	0.114
Tetracladium marchalianum	0.696	**0.019**
Lemonniera terrestris	0.854	0.396
Alatospora acuminata	0.967	0.884
Flagellospora curvula	0.984	0.687
Neonectria lugdunensis	0.997	0.986
Clavatospora longibrachiata	1	0.95
Heliscella stellata	1	0.991
Articulospora tetracladia	1	0.936
Treatment	**alder-V x alder-P**	
Tricladium angulatum	0.385	**0.039**
Neonectria lugdunensis	0.73	**0.001**
Tetracladium marchalianum	0.868	0.421
Lemonniera terrestris	0.948	0.901
Alatospora acuminata	0.973	1
Clavatospora longibrachiata	0.99	**0.018**
Flagellospora curvula	1	0.998
Heliscella stellata	1	0.988
Articulospora tetracladia	1	0.943
Treatment	**alder-V x beech-V**	
Tricladium angulatum	0.443	**0.002**
Tetracladium marchalianum	0.727	**0.002**
Lemonniera terrestris	0.864	0.193
Alatospora acuminata	0.983	0.566
Neonectria lugdunensis	0.995	0.984
Clavatospora longibrachiata	0.998	0.837
Flagellospora curvula	1	1
Heliscella stellata	1	0.991
Articulospora tetracladia	1	0.944
Treatment	**alder-beech-P x alder-P**	
Articulospora tetracladia	0.653	**0.001**
Heliscella stellata	0.837	**0.051**
Lemonniera terrestris	0.884	0.971
Alatospora acuminata	0.93	0.963
Flagellospora curvula	0.967	0.533

Neonectria lugdunensis	0.994	0.732
Clavatospora longibrachiata	1	0.475
Tetracladium marchalianum	1	0.995
Tricladium angulatum	1	0.99

A.3 Exoenzyme activity

A.3.1 Material and Methods

To quantify hydrolases and oxidases activities, we use the method described by DeForest (2009) but modified for leaf litter (see Baudy et al. 2020). Hydrolases, namely β-1,4-glucosidase (BGL; EC 3.2.1.21; targeting cellulose), cellobiohydrolase (CEL; EC 3.2.1.91; targeting cellulose), β-1,4-xylosidase (XYL; EC 3.2.1.37; targeting hemicellulose), and phosphatases (PHO; EC 3.1.3.1 and 3.1.3.2; targeting phosphate esters), were measured fluorometrically using fluorescent (MUF, methylumbelliferone)-linked artificial substrates. Oxidases, namely phenol oxidase (PHE; EC 1.10.3.2; targeting lignin) and peroxidase (PER; EC 1.11.1.7; targeting lignin), were measured colorimetrically employing L-3,4-dihydroxyphenylalanine (L-DOPA).

After thawing, leaf discs were homogenized in 350 mL of SAM-5S using an Ultra-turrax® blender (IKA®-Werke GmbH and Co. KG, Germany) at 24,000 rpm. For hydrolase analyses, black flat-bottom 96-well 300-µL plates (Thermo Fisher Scientific, USA) were incubated in darkness for 1 h on a rotary shaker (model KS 15; Edmund Bühler GmbH, Germany) at 120 rpm, whereupon 10 µL 1M NaOH were added to terminate reactions and enhance fluorescence (DeForest 2009). Fluorescence was measured at 365 nm excitation and 450 nm emission using a microplate reader (Infinite 200, Tecan Group; Switzerland). Oxidases were measured in clear flat-bottom 96-well 300-µL plates (Thermo Fisher Scientific, USA), after incubation for 2 h on a rotary shaker. Absorbance was measured at 450 nm using a microplate reader. The medium containing the homogenized leaves was filtered through pre-weighed glass fiber filters (GF/6, Whatman, Dassel, Germany) and dried at 60 ºC for 24 h to determine leaf dry mass. Enzymatic activity was expressed as µmol of degraded substrate/g leaf dry mass/hour (DeForest 2009). Further details on substrate concentrations, plate layout and calculations can be found in Baudy et al. (2020).

Table S16 - Output of two-way ANOVA as run on enzyme activity data.

Enpoint	Source of variation	Df	Sum Sq	Mean Sq	F value	p value
PER	Community history	1	$1.29x10^5$	$1.29x10^5$	0.031	0.861
	Substrate	2	$1.93x10^7$	9678745	2.336	0.118
	Community history x Substrate	**2**	**$4.07x10^7$**	**$2.03x10^7$**	**4.917**	**0.016**
	Residuals	24	$9.94x10^7$	$4.14x10^6$		
PHE	Community history	1	2121555	$2.12x10^6$	0.426	0.520
	Substrate	2	$1.98x10^7$	$9.90x10^6$	1.988	0.159
	Community history x Substrate	**2**	$1.71x10^7$	$8.58x10^6$	1.723	0.200
	Residuals	24	$1.2x10^8$	$4.98x10^6$		
GBL	Community history	1	$1.07 x10^9$	$1.07x10^9$	1.376	0.252
	Substrate	2	$1.13x10^9$	$5.67x10^8$	0.731	0.492
	Community history x Substrate	**2**	$2.05x10^9$	$1.02x10^9$	1.318	0.286
	Residuals	24	$1.86x10^{10}$	$7.76x10^8$		
XYL	Community history	1	$4.35x10^7$	$4.34x10^7$	0.68	0.418
	Substrate	2	$4.37x10^7$	$2.18x10^7$	0.342	0.714
	Community history x Substrate	**2**	$1.21x10^8$	$6.03x10^7$	0.944	0.403
	Residuals	24	$1.53x10^9$	$6.39x10^7$		
CEL	Community history	1	$1.87x10^4$	$1.87x10^4$	0.034	0.855
	Substrate	2	$3.50x10^5$	$1.76x10^5$	0.323	0.727
	Community history x Substrate	**2**	$1.31x10^5$	$6.59x10^4$	0.121	0.887
	Residuals	24	$1.31x10^7$	$5.50x10^5$		
PHO	Community history	1	$2.65x10^{11}$	$2.65x10^{11}$	1.239	0.277
	Substrate	2	$1.05x10^{12}$	$5.25x10^{11}$	2.457	0.107
	Community history x Substrate	**2**	$4.87x10^{11}$	$2.43x10^{11}$	1.139	0.337
	Residuals	24	$5.13x10^{12}$	$2.14x10^{11}$		

A.3.2 Results

A distinct pattern of the overall enzymes' activity was found for each of the treatments (Fig.3). However, only the enzyme Peroxidase showed a significant interaction of community history x leaf species (p=.016; Table 2). Higher ligninolytic activity (PHE and PER) were found in all treatments conditioned by the V- compared to the P-community. Additionally, and independent of the community history, beech-associated microbes showed a higher activity of the hydrolase enzymes XYL and CEL that target hemicellulose and cellulose, respectively. On the contrary, alder-associated microbes showed a higher activity of PHO, PHE, and PER, targeting phosphate esters and lignin, respectively. In addition, XYL and CEL activity was also

higher when alder stemming in the P-communities. The opposite, a lower activity was observed for leaves previously being colonised with V- impacted microbes. Unexpectedly, oxidase enzymes responsible for the lignin degradation were higher in the presence of alder in both P and V-impacted communities. The combination of both leaf species resulted in a higher activity of hydrolases (XYL and CEL) independent of the microbial community history, and as observed for beech leaves.

References:

Gonçalves S., Feckler A., Pollitt A., Baschien C., Michael J., Schreiner V. C, Zubrod J. P., Bundschuh M., Increasing fungicide and nutrient concentrations change structure but not function of aquatic microbial communities *(submitted)*

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7.4 APPENDIX IV

Author contribuitions

Appendix I

Increasing fungicide and nutrient concentrations change structure but not function of aquatic microbial communities

Conceptualisation: MB.

Conducting the research: SG; AP; JM; VS; AF; CB.

Data analysis: SG; CB; AF; JZ.

Data interpretation: SG; CB; AF; MB.

Preparation figures & tables: SG.

Writing: all.

Appendix II

Leaf Species-Dependent Fungicide Effects on the Function and Abundance of Associated Microbial Communities.

Conceptualization: MB, JZ.

Methodology:RP, MK, JZ.

Formal analysis and investigation: SG, RP, AF.

Writing and original draft preparation: SG.

Writing, review and editing: all.

Funding acquisition: MB.

Appendix III

Microbial community history and leaf species shape bottom-up effects in a freshwater shredding amphipod

Conceptualisation: MB.

Developing methods: SP.

Conducting the research: SG; AP; SP; AF.

Data analysis: SG.

Data interpretation: SG, AP, AF, MB.

Preparation of figures & tables: SG.

Writing: all.

7.5 APPENDIX V - Curriculum vitae

O seu nome

Skills:

Laboratory (enzymatic assays, cell culture, qPCR, Microscopy)
Microsoft office
R studio basics
Field work

Languages:

Native speaker
C1
B1
A1

Professional experience

→ 05/2023 – present
Research assistant – *TIP* project, Department of Environmental Toxicology, Eawag - Swiss Federal Institute of Aquatic Science and Technology, Duebendorf, Switzerland

→ 02/2019 – present
PhD student (BIO2FUN project funded by DFG) at iES Landau, RPTU Kaiserslautern – Landau, Germany

→ 02-08/2016, 10-12/2017, 09-11/2018
Research assistant at Aquatic Sciences and Assessment Department, Swedish University of Agricultural Sciences, SLU Uppsala, Sweden

→ 08-10/2015, 10/2016-01/2017
Erasmus + Traineeships - Aquatic Sciences and Assessment Department, SLU Uppsala, Sweden

→ 09/2014 – 05/2015
Erasmus + Traineeship – INRA-Carrtel station, Thonon, France & Department of Biology, University of Aveiro, Portugal

Education

→ 2015-2017 Msc Cell and Molecular biology. Department of Biology, University of Aveiro, Portugal
Final average: 17.4 points out of 20
Msc thesis: Zinc and Copper impacts on freshwater diatoms: physiological, biochemical and metabolomic response of *Tabellaria flocculosa* - 19 points out of 20

→ 2011-2015 Bsc Biology. Department of Biology, University of Aveiro, Portugal
Final average: 15 points out of 20
Bsc thesis: Effects of metals on diatoms' biochemistry – 19 points out of 20

Peer-reviewed publications

Gonçalves, S., Baschien, C., Feckler, A., Bundschuh, M. (**in prep**). *qPCR & NGS methods to study leaf litter community composition under stress.*

Gonçalves, S., Feckler, A., Pollitt, A., Pietz, S., Schreiner, V. C., Bundschuh, M. (**in prep**). *Individual traits of aquatic hyphometes under fungicide and nutrient stress*

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Conference contributions (presenting author)

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S. Gonçalves, A. Pollitt, A. Feckler, M. Bundschuh, Dietary Effect Pathway in *Gammarus fossarum* (Crustacea ;Amphipoda): Influence of Land-Use and Leaf Substrate. Platform presentation, SETAC Europe 32th Annual Meeting – Copenhagen 2022

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