Science of the Total Environment 533 (2015) 40–48

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of fungicides on decomposer communities and litter decomposition in vineyard streams

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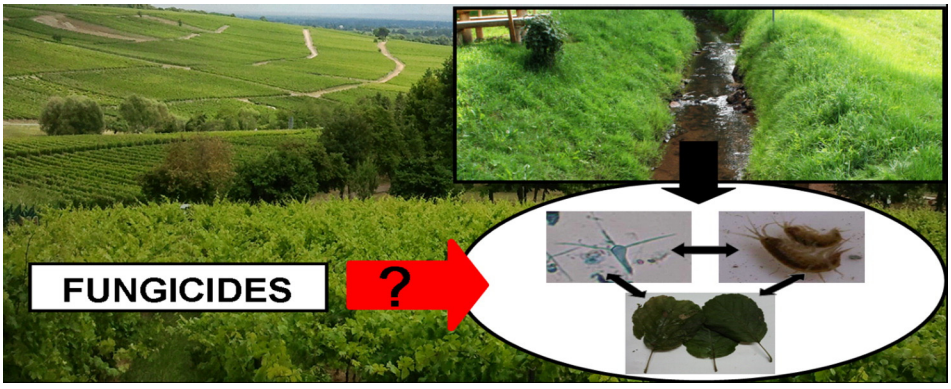
HIGHLIGHTS

- We conducted a field study in 17 fungicide-exposed vineyard streams.
- Microbial and shredder communities changed along the fungicide toxicity gradient.
- Microorganism-mediated decomposition decreased up to 40% in polluted streams.
- Invertebrate-mediated decomposition was negatively associated with sediment copper.
- Additional laboratory experiment suggested causality of fungal community changes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 22 June 2015
Accepted 22 June 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Pesticide
Leaf breakdown
Shredder
Microorganism
Aquatic toxicity

ABSTRACT

Large amounts of fungicides are applied globally and partly enter freshwater ecosystems. A few laboratory studies examined their effects on decomposer communities and the ecosystem process of litter decomposition (LD), whereas the field situation remains largely unknown. We conducted a field study with 17 stream sites in a German vineyard area where fungicides represent the dominant pest control agent. Passive samplers were used to monitor 15 fungicides and 4 insecticides in streams and their toxicity was described using the toxic unit approach, whereas sediment samples were taken to characterise total copper concentrations. Microbial and leaf-shredding invertebrate community composition and related LD rates were assessed at each site. The structure of microbial and shredder communities as well as fungal biomass changed along the fungicide toxicity gradient. The changes in microbial endpoints were associated with a reduction of microbial LD rate of up to 40% in polluted streams. By contrast, neither the invertebrate LD rate nor in-situ measured gammarid feeding rates correlated with fungicide toxicity, but both were negatively associated with sediment copper concentrations. A subsequent laboratory experiment employing field fungicide concentrations suggested that the microbial community changes are causal. Overall, our results suggest that fungicides can affect LD under field conditions.

1. Introduction

Human activities exert high pressure on freshwater ecosystems. Among the different stressors, pollution has been identified as a major driver of freshwater biodiversity loss (MEA, 2005; Vörösmarty et al.,

Abbreviations: LD, litter decomposition; TU, Toxic Units; SumTU, logarithmic sum of Toxic Units; *k*, leaf decomposition rate.

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http://dx.doi.org/10.1016/j.scitotenv.2015.06.090
0048-9697/

2010) consequently threatening ecosystem health and the provisioning of ecosystem processes and services (Dudgeon et al., 2006). In this context, pesticides contribute substantially to freshwater pollution because large amounts are applied worldwide and partly enter surface waters (Köhler and Triebskorn, 2013; Schwarzenbach et al., 2010) with rainfall-triggered runoff as a major input path (Leu et al., 2004a). When entering a stream, they may affect the different groups of organisms involved in allochthonous coarse litter decomposition (LD) which in turn might propagate to a reduction of this ecosystem process (Peters et al., 2013; Schäfer et al., 2007). LD plays a key role in stream ecosystems, because it represents the dominant energy and nutrient source for the heterotrophic food web (Wallace et al., 1997), particularly in headwaters (Fisher and Likens, 1973). In addition, dominance of allochthonous inputs over primary production can extend up to tens of kilometres from headwaters (Webster, 2007). Considering that 50% of the approximately 1.2 million km of the stream network (scale 1:250,000) in Europe are small rivers and streams (Globevnik, 2007) and that heterotrophic metabolism dominates in low-order streams (Vannote et al., 1980), allochthonous inputs are of central importance. In this context, fungi and bacteria are the main microbial decomposers converting organic matter into a more nutritious food resource for leaf-shredding macroinvertebrates (Gessner et al., 2007). Despite the fact that fungicides are the most heavily used group of pest control agents in regions such as the European Union and that they can affect non-target freshwater fungi (Dijksterhuis et al., 2011), they represent the least studied group of pesticides (Köhler and Triebskorn, 2013). Under laboratory conditions, fungicides change fungal community structure and decrease fungal biomass (Bundschuh et al., 2011b), which resulted in a decrease in microbial LD (Artigas et al., 2012; Rasmussen et al., 2012a). Moreover, shredders showed preference for non-exposed leaves in comparison to leaves that have been exposed to fungicides (Bundschuh et al., 2011b) and were directly affected by fungicides at environmentally relevant concentrations (Flores et al., 2014; Zubrod et al., 2014). However, it remains open whether and to which extent these studies can be extrapolated to the field situation, where multiple factors such as biotic interactions, abiotic factors and recolonisation may moderate the effects of fungicides on LD.

In this study we assessed the effects of fungicides on LD and the associated decomposer communities in 17 streams within a vineyard area where fungicides are the dominant pest control agent. Microbial and leaf-shredding invertebrate communities were characterised together with microbial and invertebrate-mediated LD in autumn 2012 (Supplementary data Fig. S1), coinciding with the major leaf litter input into streams. Fungicide exposure in stream water was monitored the same year during four rainfall events in summer and early autumn, covering the main fungicide application period. At the same time, the ecotoxicological potential of the pesticide loads introduced during these run-off events was empirically estimated by means of in-situ bioassays using gammarid feeding as endpoint. Moreover, the toxicity of each pesticide for microorganisms and invertebrates at the different sampling sites was assessed using Toxic Units (Ohe and Zwart, 2013) and subsequently aggregated per site and rainfall event. Total copper in stream sediment was also assessed because of its use as fungicide in vineyards, where it tends to accumulate in the soil and reaches the stream mainly via physical erosion (Bereswill et al., 2012). Finally, a laboratory experiment was conducted to identify whether correlations between microbial community change and fungicide toxicity are causal. We hypothesised that fungicide toxicity would lead to a shift in the fungal community and a decrease in fungal biomass and that these changes would be associated with a reduction in microbial and invertebrate LD.

Table 1
Environmental variables characterising the 17 sampling sites included in this study.

Variable	Minimum	Maximum	Median	Mean	SD
Stream width (m)	0.80	7.30	1.67	2.21	1.61
Stream depth (m)	0.07	0.43	0.15	0.19	0.10
Current velocity (m/s)	0.01	0.67	0.23	0.26	0.17
Temperature (°C)	11.21	13.77	12.62	12.50	0.81
pH	7.51	8.26	7.87	7.85	0.24
Oxygen (mg/L)	5.30	10.61	9.60	9.10	1.30
Conductivity (μS/cm)	110	1290	332	481	340
Nitrite (mg/L)	0.00	0.80	0.04	0.09	0.19
Nitrate (mg/L)	2	60	5	9	14
Phosphate (mg/L)	0.10	0.60	0.20	0.25	0.13
Ammonium (mg/L)	0.00	0.20	0.00	0.01	0.05
Riffle sections (%)	0	100	80	70	36
Pool sections (%)	0	100	20	30	36
Leaves and wood (<10 cm diameter)[a]	1	3	1	–	–
Wood (>10 cm diameter)[a]	1	2	1	–	–
Filamentous algae[b]	0	2	0	–	–
Macrophytes[b]	0	2	0	–	–
Shading[b]	2	5	4	–	–
WRF (width of the riparian forest; m)[c,d]	1	7.5	2.25	–	–
Tree cover along the bank[b,d]	0.5	5	2.5	–	–
Large substrates (boulder + cobble; %)	0	100	20	31	31
Medium substrates (pebble + gravel; %)	0	60	5	19	23
Fine substrates (sand + silt; %)	0	100	35	50	39
$SumTU_{P.\ subcapitata}$	−4.87	−2.02	−3.73	−3.66	1.07
$SumTU_{D.\ magna}$	−5.34	−1.87	−3.86	−3.91	1.25

[a] Measured using an ordinal scale indicating the coverage, ranging from 1 (<5%) to 3 (>20%).
[b] Measured using an ordinal scale indicating the coverage, ranging from 0 (absent) to 5 (very high).
[c] Width of the riparian forest. When riparian forest was connected with the hillside forest, a maximum of 10 m was assigned.
[d] Mean from the left and right banks.

2. Methods

2.1. Study area

The study was conducted in the wine-growing area of Palatinate (southwest Germany, Supplementary data Fig. S2), which covers more than 23,000 ha of vineyards (Statistisches Landesamt RLP, 2011). Fungicides are applied every 10–14 days from end of April to mid August and are the most used pesticides for grapes (96% of all applications), whereas herbicides (1.5%) and insecticides/acaricides (2.5%) play a minor role (Roßberg, 2010), the latter ones owing to the use of pheromone traps. All streams originate in the Palatinate Forest Nature Park, a forested low-mountain range. After the forest, they discharge through an agricultural landscape that is dominated by vineyards in the first kilometres. We selected 17 sampling sites in different streams covering a presumed gradient of fungicide exposure based on the proportion of vineyards in the upstream catchment (Supplementary data Fig. S2). Four sites were located upstream from vineyards in the forest and served as reference sites without exposure. Sites were selected to exclude other major pressures upstream (such as large waste water treatment plants, large urban areas and industrial facilities) to allow for an identification of potential impacts from fungicides. Moreover, the sampling sites were located within a maximum distance of four km to the forest edge to allow for potential recovery of the invertebrate community from previous effects (Hatakeyama and Yokoyama, 1997; Liess and Ohe, 2005).

2.2. Environmental parameters

Physico-chemical data was collected in concert with leaf deployment at each site using the Rapid Bioassessment Methodology for Rivers and Streams (EPA, 2003; for parameters see Table 1). 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