Aquatic Toxicology 186 (2017) 215–221

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Interactive effects of an insecticide and a fungicide on different organism groups and ecosystem functioning in a stream detrital food web

Mohab Dawoud[a,b], Mirco Bundschuh[a], Willem Goedkoop[a], Brendan G. McKie[a,*]

[a] Department of Aquatic Sciences & Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Low Emission Capacity Building Project, 53 Misr- Helwan Elzerayea Rd., Lo'Lo'at Elmaadi Tower, Entrance C, Floor 5, Maadi, Cairo, Egypt

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form 3 March 2017
Accepted 7 March 2017
Available online 9 March 2017

Keywords:
Ecosystem processes
Invertebrates
Fungi
Microbes
Multi-stressor interactions
Non-target organisms
Pesticides
PICT

ABSTRACT

Freshwater ecosystems are often affected by cocktails of multiple pesticides targeting different organism groups. Prediction and evaluation of the ecosystem-level effects of these mixtures is complicated by the potential not only for interactions among the pesticides themselves, but also for the pesticides to alter biotic interactions across trophic levels. In a stream microcosm experiment, we investigated the effects of two pesticides targeting two organism groups (the insecticide lindane and fungicide azoxystrobin) on the functioning of a model stream detrital food web consisting of a detritivore (Ispoda: *Asellus aquaticus*) and microbes (an assemblage of fungal hyphomycetes) consuming leaf litter. We assessed how these pesticides interacted with the presence and absence of the detritivore to affect three indicators of ecosystem functioning – leaf decomposition, fungal biomass, fungal sporulation – as well as detritivore mortality. Leaf decomposition rates were more strongly impacted by the fungicide than the insecticide, reflecting especially negative effects on leaf processing by detritivores. This result most like reflects reduced fungal biomass and increased detritivore mortality under the fungicide treatment. Fungal sporulation was elevated by exposure to both the insecticide and fungicide, possibly representing a stress-induced increase in investment in propagule dispersal. Stressor interactions were apparent in the impacts of the combined pesticide treatment on fungal sporulation and detritivore mortality, which were reduced and elevated relative to the single stressor treatments, respectively. These results demonstrate the potential of trophic and multiple stressor interactions to modulate the ecosystem-level impacts of chemicals, highlighting important challenges in predicting, understanding and evaluating the impacts of multiple chemical stressors on more complex food webs *in situ*.

1. Introduction

The development of the "Green revolution" during the 20th century dramatically raised agricultural production, especially through the extensive use of fertilizers and pesticides (Tilman, 1998). Such pesticides comprise a wide variety of insecticides, herbicides and fungicides, targeting different pest organism groups on crops. During or following their application onto agricultural fields, pesticides can be transferred into adjacent aquatic ecosystems, especially via spray drift or surface runoff (Schulz, 2004). Once there, these "pesticide cocktails" may affect the structure and diversity of aquatic communities (Schulz and Liess, 1999), and also key ecosystem processes such as algal productivity (Villeneuve et al., 2011) and leaf litter decomposition (Schafer et al., 2007). These impacts arise not only from the potential for direct interactions among the pesticides, but also from "knock-on" (i.e. secondary indirect or cumulative) effects of those interactions on organisms and the ecosystem functions they perform (Norgaard and Cedergreen, 2010; Truchy et al., 2015).

The decomposition of terrestrially-derived leaf litter is a key process in the functioning of streams, involving multiple organism groups and trophic levels. Leaf litter decomposition in streams begins with the leaching of soluble compounds from freshly shed litter, followed by colonization of microbes, particularly aquatic hyphomycete fungi (Gessner et al., 1999). Microbial colonization facilitates leaf degradation through enzymatic hydrolysis that converts organic matter to CO_2 and biomass (Gessner et al., 2010). This process, also known as "microbial conditioning", increases the palatability of the litter for leaf-consuming detritivores (a.k.a. "shredders") (Bärlocher, 1985; Cummins and Klug, 1979), which are responsible for the bulk of the physical fragmentation of leaves

---

[*] Corresponding author at: Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, 750 07 Uppsala, Sweden.
E-mail address: Brendan.Mckie@slu.se (B.G. McKie).

http://dx.doi.org/10.1016/j.aquatox.2017.03.008

into fine particulate organic matter (Anderson and Sedell, 1979; Hieber and Gessner, 2002).

Due to the interconnected nature of stream food webs, pesticides targeting one group of organisms can have knock-on effects on other trophic levels and organism groups (Bundschuh and McKie, 2016). Such effects can propagate both "bottom-up", where effects on the composition and activity of organisms at the base of the food web impact higher trophic levels, and "top-down", where impacts on higher trophic levels (i.e. primary or secondary consumers) strongly influence lower trophic levels (Gotelli and Ellison, 2006; Jabiol et al., 2013). For example, fungicides that impact biomass and activities of microbes are likely to also impair microbial conditioning and thereby reduce detritivore feeding, further reducing overall rates of leaf decomposition (Fernandez et al., 2015; Gardeström et al., 2016). Insecticides can impact decomposition mediated through effects on the feeding behavior, abundance (mortality) and/or diversity (selective mortality) of invertebrate detritivores (Schafer et al., 2007). However, impacts of insecticides on leaf processing by microbes are difficult to predict (Chung and Suberkropp, 2008) – given that detritivores themselves consume microbes, a negative effect on detritivore feeding activity may even favor greater microbial activity (Graça et al., 1993). Unraveling the individual and joint consequences of pesticides targeting different types of organisms might aid our understanding of the relevance of the different functions provided by these trophic levels for the ecosystem process of, for example, leaf litter decomposition.

We investigated the effects of two pesticides (a fungicide and insecticide) on the functioning of a tritrophic (detrital resource – microbial decomposers – invertebrate detritivore) stream food web in laboratory microcosms, as a model system for investigating complex multiple stressor and -trophic interactions. In a factorial experiment, these microcosms were subjected either to (i) a pesticide free control, (ii) an insecticide only treatment, (iii) a fungicide only treatment, or (iv) a combined pesticide treatment with the fungicide and insecticide applied jointly. The presence or absence of the detritivore *Asellus aquaticus* (Crustacea: Isopoda) was also varied among microcosms. Our response variables included rates of leaf mass loss, the mortality and leaf processing efficiency (LPE) of detritivores, as well as the biomass and sporulation activity of fungi. We hypothesized that the fungicide as single stressor would affect microbially-mediated decomposition, fungal biomass and fungal sporulation activity negatively, which may have knock-on effects arising from the bottom-up on the responses of detritivores, reflecting impaired microbial conditioning. In contrast, we expected that the insecticide would affect the detrital food web from top-down, by reducing LPE of detritivores, but possibly enhancing some microbial responses due to a reduction in detritivore feeding pressure. Finally, we hypothesized that the joint application of the fungicide and insecticide would reflect the combined impacts of impaired microbial activity caused by the fungicide and impaired detritivore feeding caused by the insecticide.

2. Materials and methods

2.1. The pesticide treatments

Our selected model pesticides were the fungicide azoxystrobin and insecticide lindane. Azoxystrobin is primarily used to prevent foliar diseases of vegetable and fruit crops caused by pathogenic fungi (Ascomycota, Deutermyctoa, Basidomycota) and fungi-like organisms (Oomycetes) (Bartlett et al., 2002). Azoxystrobin affects fungal reproduction and development by disturbing the energy production for spore germination and zoospore motility (Bartlett et al., 2002). Azoxystrobin is frequently detected in agricultural streams (Bereswill et al., 2012) and has the potential to alter the structure and function of aquatic leaf-associated microbial communities (Gardeström et al., 2016; Zubrod et al., 2015). Hence, this fungicide is selected as model chemical stressor impacting the detrital food web primarily from the bottom-up.

Lindane, in contrast, represents organochlorine pesticides, which inhibit in the nervous systems of insects (Stenersen, 2004), and are thus most likely to impact the functioning of the detrital food web from the top-down. Lindane is applied to a wide range of crops, targeting soil-dwelling and plant-eating invertebrates. Although lindane is banned in Europe, it continues to occur in European water bodies at low ng/l levels, reflecting ongoing deposition from rainwater and runoff from legacy deposits in soils (e.g., Nanos et al., 2012).

Stock solutions of these pesticides were prepared from analytical standards in acetone (lindane: 500 μg/ml; azoxystrobin: 5200 μg/ml; and the lindane-azoxystrobin combination: 500 + 5200 μg/ml). Pesticides were introduced from these stock solutions into the test medium (50 ml per replicate), i.e. the standard test medium M7 (OECD, 1998). The resultant pesticide concentrations (azoxystrobin at 2600 μg/l; lindane at 5 μg/l) were at non-lethal levels for *A. aquaticus* based on published work (Gardeström et al., 2016; Le Bras, 1984). As acetone itself may affect biota, we established an extra set of eight microcosms (four with *A. aquaticus* and four without; see details below), which were spiked with 50 μl of pure acetone and thereby served as solvent control. However, there were no statistically significant differences between the control and solvent control microcosms for any of our response variables, when compared either as a main effect (ANOVA all $F < 2.9$, all $p > 0.11$) or in interaction with the *A. aquaticus* treatment (ANOVA all $F < 2.6$, all $p > 0.13$). Therefore, these data are not further considered in the present study.

2.2. Leaf litter and fungal colonization

Alnus glutinosa leaf litter was collected just prior to abscission near Uppsala (59°48′55.95″N, 17°40′11.46″E) during October 2010, and subsequently air-dried in the laboratory. Prior to the experiment, these leaves were rewetted in the test medium, with leaf discs subsequently cut using a 15 mm cork borer, to standardize leaf surface area. The central leaf vein, which is of low nutritional value, was avoided when cutting the leaf discs.

Rather than colonize the leaf discs with hyphomycete fungal spores directly in the field, the discs were colonized from an additional set of pre-conditioned leaves in the laboratory (Bundschuh et al., 2011; Gardeström et al., 2016). This avoids variability in both fungal community composition and litter decay state potentially associated with microhabitat variability in the field, ensuring a greater standardization in the condition of the discs prior to the experiment. Colonization was achieved via a two-stage protocol: An additional set of whole leaves was exposed in fine mesh bags (1 mm mesh size) in a nearby stream (Hågaån, 59.80°51′30″N, 17.61°39′0″E), which has a mixed forested and agricultural catchment (Gardeström et al., 2016). The leaves were exposed for three weeks during April 2011, to allow colonization by fungi. The field-colonized litter was later transferred to laboratory aquaria. Subsequently, the leaf discs were evenly distributed among four polyamide mesh bags (15*15 cm, mesh size 0.5 mm), which were then immersed within the aquaria. The discs were left for fourteen days, sufficient for development of a diverse and abundant community of fungi (Gardeström et al., 2016).

2.3. Detritivore collection

Asellus aquaticus is a leaf-shredder that is relatively tolerant of degraded environments, and often abundant in the agricultural streams of Europe (Carlson et al., 2013; Hladyz et al., 2011). One week prior to the experiment in May 2011, 140 individuals between