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Effects of a fungicide (imazalil) and an insecticide (diazinon) on stream fungi and invertebrates associated with litter breakdown

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HIGHLIGHTS

- We studied the effects of imazalil and diazinon on freshwater organisms associated with leaf breakdown.
- Pesticides altered sporulation and fungal community composition.
- Invertebrate death rates increased and body condition decreased under the effect of pesticides.
- Pesticides can disrupt organic matter processing and energy cycling in streams.
- Longer exposure times can lead to stronger alterations of freshwater ecosystems.

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ABSTRACT

The intensification of agriculture has promoted the use of pesticides such as fungicides and insecticides. Many pesticides readily leach into natural water bodies and affect both organisms and ecosystem processes such as leaf breakdown, a crucial process in headwater streams. As leaf breakdown in streams involves sequential steps by different groups of organisms (first microbial conditioning, then invertebrate shredding), pesticides targeting different organisms are likely to affect one or the other step, and a mixture of contaminants might have interactive effects. Our objective was to evaluate the effect of a fungicide (imazalil) and an insecticide (diazinon) on stream fungal and invertebrate activities, and their effects on leaf consumption. After an initial assay to define 'effective concentration' of both pesticides in a laboratory experiment, we manipulated pesticide presence/absence during the conditioning and shredding phases. Both pesticides affected fungal community and reduced the performance of the shredding amphipod *Echinogammarus berilloni*, and leaf consumption. The impact of pesticides on fungal sporulation depended on the length of the exposure period. In addition, pesticides seemed to cause an energetic imbalance in the amphipod, affecting body condition and mortality. The combined effect of both pesticides was similar to those of the fungicide. Overall, our results show that the effects of pesticide mixtures on leaf breakdown are hard to predict from those observed in either fungi or macroinvertebrate performance.

1. Introduction

Population growth and intensification of human activities in the recent decades have led to such a dramatic transformation of the Earth in a truly global environmental change (Vitousek, 1997), that Crutzen (2002) coined the term "Anthropocene" to refer to the current era. Among many other environmental changes, agricultural land use has greatly increased in cover and intensity (Allen and Barnes, 1985; Foley et al., 2005). This has been matched by increased use of fertilizers and pesticides worldwide (Tilman, 1998), often threatening the receiving aquatic ecosystems (Stoate et al., 2001; Vörösmarty et al., 2010; Sutton et al., 2011).

Pesticides enter water bodies via different pathways, including diffuse inputs from spraying, runoff from agricultural lands (Barth et al., 2008), and outflows from wastewater treatment plants (Gerecke et al., 2002) and contaminated sediments (Bermúdez-Couso et al., 2007). Many can even travel long distances in the atmosphere (Bartrons et al., 2012) following what has been called the global distillation (Goldberg, 1975; Wania and Mackay, 1996). These substances often have potent biological effects, and their presence in the environment raises such serious concerns (Fleeger et al., 2003; Schulz, 2004), that many of them have been included in legislation since 1979 (e.g. European Commission, 1991, 91/414/EEC; European Commission, 1998, 98/8/EC). Pesticides can reduce biodiversity (Beketov et al., 2013) and production of microbial and invertebrate biomass (Artigas

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et al., 2012; Rasmussen et al., 2012), and may disrupt ecosystem functioning (Schäfer, 2012; Schäfer et al., 2012a) that underpin many ecosystem services (Costanza et al., 1997). Ecosystem functioning is governed by dynamic biophysical processes such as nutrient retention or oxygen production, and is a key component of ecosystem health (Bunn et al., 1999; Young et al., 2008). Therefore, it includes a wide variety of processes that can be assessed by different metrics (Palmer and Febria, 2012), varying in the temporal and spatial scales at which they respond to a given environmental stressor (Elosegi and Sabater, 2013).

Organic matter breakdown is an essential ecosystem function in all streams which has been considered an appropriate indicator of their functional integrity (Gessner and Chauvet, 2002) because it responds in predictable ways to factors such as the concentrations of nutrients and pollutants in water (Aristi et al., 2012; Woodward et al., 2012). It is a complex process that involves leaching, physical abrasion, colonization by microbes, and shredding of organic matter by invertebrates (Gessner et al., 1999; Tank et al., 2010). Consequently it is sensitive to toxins affecting the different groups of organisms (fungi, bacteria and invertebrates) involved in breaking down organic matter. Furthermore, there is a positive feed-back between the microbes that increase the resource quality for consumers and the shredding invertebrates that fragment detritus what enhances its surface-to-volume ratio, thereby promoting further microbial colonization (Bergfur et al., 2007; Greenwood et al., 2007). In addition to direct effects on their target organisms, pesticides entering streams likely affect non-target groups and thus, organic matter breakdown in a complex way, although there is so far little experimental evidence (Rohr et al., 2006).

Although monitoring by water agencies and researchers has greatly increased the information on the distribution and concentration of pesticides in rivers (Lacorte et al., 2006; EEA, 2012), there is still little knowledge on their interactive effects in complex mixtures as those commonly found in rivers (Kuster et al., 2008). In a review of pesticides in freshwater systems, Relyea and Hoverman (2006) found that only 8% of the studies examined the effects of pesticide mixtures, despite the fact that interactions among substances often modify the effects of individual compounds on living organisms (Vinebrooke et al., 2004). Although there are some recent studies regarding the effects of pesticides on the community structure of stream organisms (Liess and Von Der Ohe, 2005; Beketov et al., 2013), there is far less information on the interactive effects of pesticides on river ecosystem functioning. For example, Peters et al. (2013) reported that functioning of lake and river ecosystems was affected by concentrations of toxicants that were below toxic levels set for standard test organisms (the cladoceran *Daphnia magna* and the green algae *Pseudokirchneriella subcapitata*) but the study did not show a direct relationship between contaminant toxicity and ecosystem function.

Our objective was to test the effects of a fungicide (imazalil) and an insecticide (diazinon), singly and combined, on aquatic organisms related to leaf litter breakdown. As both tested pesticides have different modes of action and target organisms, the effects were tested on two phases of the leaf breakdown process (i.e. leaf conditioning, shredding or both). We predicted that a) both pesticides would adversely affect breakdown, when fungi are exposed to imazalil during the conditioning phase and when the amphipods are exposed to diazinon during the shredding phase; b) the effects on leaf consumption would be highest when both pesticides are present during conditioning and shredding as effects on both organism groups would be additive; c) the two pesticides would have synergistic interactions in mixture, and thus lead to higher alterations than single pesticide treatments.

2. Methods

2.1. Chosen pesticides and organism species

Our interest was to test the effect of two pesticides commonly found in streams on two freshwater organisms associated with organic matter breakdown. Imazalil and diazinon are two pesticides commonly found in streams and rivers (Castillo et al., 2006; Gómez et al., 2012). In rivers of the Iberian Peninsula, diazinon has been reported in a range of concentrations between 12 and 450 ng L^{-1} and imazalil between 5 and 2100 ng L^{-1} (Campo et al., 2013; Masiá et al., 2013). Nevertheless, concentrations of imazalil up to 160 μg L^{-1} were reported in a stream draining a banana plantation (Castillo et al., 2006). Imazalil ($C_{14}H_{14}C_{l2}N_2O$) is a systemic fungicide that affects cellular membrane permeability by inhibiting ergosterol synthesis, and is used as an agricultural fungicide against fungal pests in fruit and vegetable crops (FAO, 2001). It is stable for at least 8 weeks at 4.5 °C, its half-life in the field is 150 days and its water solubility is 180 g L^{-1} (Haarstad et al., 2012). Diazinon ($C_{12}H_{21}N_2O_3PS$) is an organophosphate insecticide that inhibits acetylcholinesterase and other enzymes with a similar structure, affecting connexions of the nervous system. This compound is widely used for agricultural, commercial, and especially domestic purposes (Cox, 2000). Its solubility in water is 60 g L^{-1} The half-life of diazinon is estimated to be 87 days in the aqueous phase, but only 3.9–4.7 days in aerobic laboratory natural sediment water system experiments (EFSA, 2006). For *D. magna*, the 48 h EC_{50} of imazalil and of diazinon are 2.6 mg L^{-1} and 0.7 μg L^{-1}, respectively. Both pesticides (Sigma-Aldrich; >97% purity) were dissolved in water collected from an unpolluted stream near the Faculty of Science and Technology (University of the Basque Country) with a pH of 8.16 and 517 μs cm^{-1} of conductivity.

We selected the amphipod *Echinogammarus berilloni* instead of an insect primarily due to the abundance of this shredder in North Iberian streams (Larrañaga et al., 2009). On the other hand, we wanted to estimate the effects of pesticides in processes at the ecosystem level, and that might imply their effect on non-target, but abundant, organisms. Ovigerous females of *E. berilloni* were selected to ensure that all individuals were mature. Organisms were collected from the same stream where water was collected and where leaves had been pre-colonized (see below).

2.2. Experimental design

Two consecutive laboratory experiments were carried out to assess the effects of imazalil and diazinon on fungal communities, invertebrate performance and leaf consumption. The objective of the first experiment was to determine 'effective concentrations' (i.e. a concentration that induces a measurable response on the organisms) of each pesticide for aquatic fungi and for *E. berilloni*. The second experiment was carried out to assess the effects of the pesticides singly or together and the relevance of the phase of the decomposition process affected (i.e. conditioning or shredding). Both experiments were carried out in laboratory chambers at 10 °C and a photoperiod of 8 h light–16 h dark.

Freshly fallen leaves of alder (*Alnus glutinosa*) were enclosed in 0.5-mm mesh bags and incubated in a non-polluted stream near the Faculty of Science and Technology (University of the Basque Country) for 2 weeks to allow fungal colonization. The leaves were then collected, rinsed with tap water in the laboratory to remove invertebrates, and conditioned for a week in glass tanks with stream water and the required pesticide treatment. This was called the conditioning phase, and was repeated every week to provide fresh food for the invertebrates (see Gantt chart in Supplementary material). After conditioning, 12-mm diameter discs were cut from the leaves, and groups of three discs were fed to *E. berilloni* that were individually enclosed in 250 mL water glasses. This corresponded to the shredding phase, and 15 individual amphipods were assigned to each treatment (i.e. 15 replicates per treatment). Food and water were renewed every 7 days with the corresponding pesticide concentration and conditioned leaf type.

In the first experiment, leaves (during the conditioning) and shredders (during the shredding phase) were exposed to the same nominal pesticide concentrations: 0, 0.1, 1, 10 and 100 μg L^{-1} for imazalil (fungicide) and 0, 0.05, 0.5, 5 and 50 μg L^{-1} for diazinon (insecticide).